

THE INHERITANCE OF THE FLOWER
COLOUR AND THE SEED COLOUR IN
LUPINUS ANGUSTIFOLIUS

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CARL HALLQVIST

LIC. PHIL., LD.

LUND 1921, BERLINGSKA BOKTRYCKERIET

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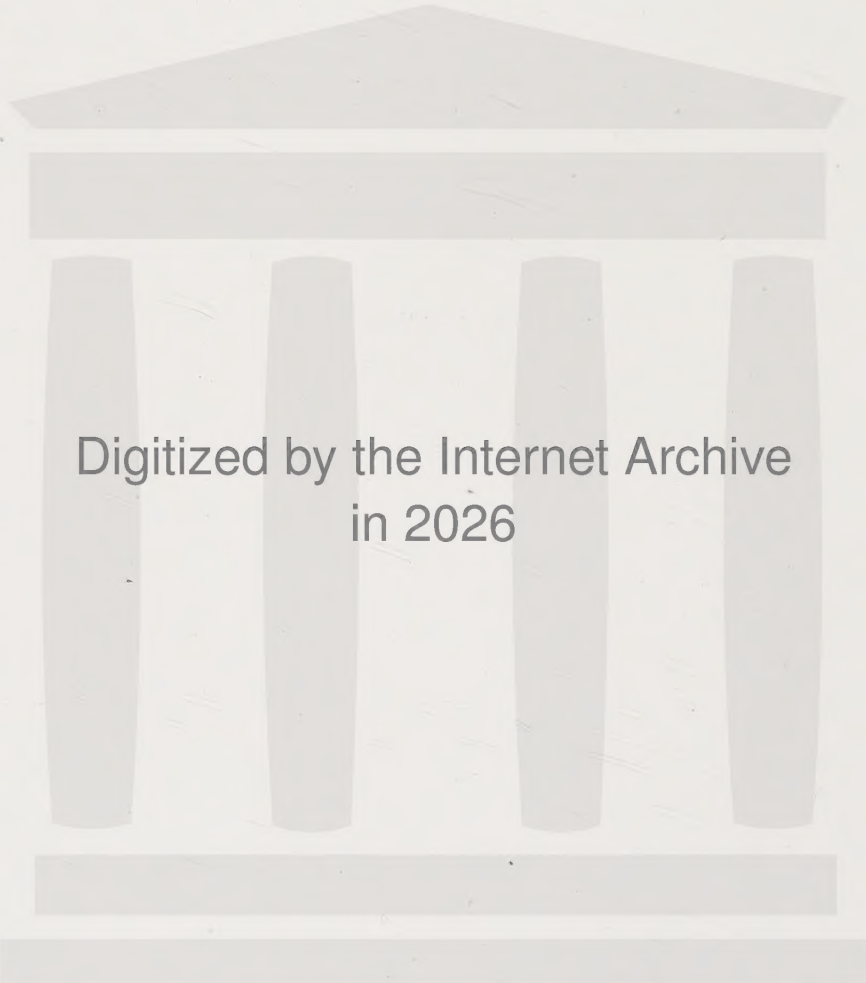
BY DUE PERMISSION OF THE PHILOSOPHICAL FACULTY OF THE UNIVERSITY OF LUND
TO BE PUBLICLY DEFENDED IN THE BOTANICAL AUDITORY,

MAY 9, 1921, AT 12 O'CLOCK,

FOR THE DEGREE OF DOCTOR OF PHILOSOPHY



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THE INHERITANCE OF THE FLOWER COLOUR AND THE SEED COLOUR IN LUPINUS ANGUSTIFOLIUS

BY CARL HALLQVIST
WEIBULLSHOLM, LANDSKRONA

SEVERAL types of flower colour and seed colour are known in *Lupinus angustifolius* since long ago. The data have been brought together in »Die Züchtung der landwirtschaftlichen Kulturpflanzen», where FRUWIRTH (1919) mentions the occurrence of blue, light blue, rose (probably identical with my bluish red) and white flower colours. It is also stated here that dark seed colour and coloured flowers are correlated, as well as white seed colour and white flowers. FRUWIRTH hybridized blue and white types and found that blue flower colour and dark coloured, marbled seeds were dominant over white flower colour and white seeds. In F_2 the hybrids segregated in the ratio 3 : 1.

According to HARZ (in Landwirtschaftliche Samenkunde) there is also found a variety named *leucospermum* with coloured flowers and white seeds. The existence of this variety has been doubted by KAJANUS. According to him, the cause of proposing this variety must be due to the occurrence of white seeds in samples of blue flowered varieties. The fact that white flowers become coloured at a later stage (see pag. 302) may also account for the fact, especially since the intensity of this colour varies greatly in response to varying environmental conditions. No type with white seeds combined with coloured flowers in earlier stages has been found in my material.

The most complete investigation hitherto made of the inheritance of the flower colour in *Lupinus angustifolius* has been published by VESTERGAARD (1919). He also finds that the blue and white colours segregate in the ratio 3 : 1. He also crossed red and white types and succeeded in obtaining a synthesis of the blue colour in F_1 and F_2 , which segregated regularly in 9 blue, 3 red and 4 white. The red type in VESTERGAARD's cultures is probably also identical with my bluish red.

The seed colour has been investigated by FRUWIRTH (1915) and KAJANUS (1912). FRUWIRTH has obtained a type with dark, self-coloured seeds. He has studied its inheritance, and he has arrived at very peculiar results. I have had no type of this kind in my material.

KAJANUS has classified different types of seed colour, but no study of their inheritance has been made by him. I am much indebted to KAJANUS for receiving material of the different types of seed and flower colours.

THE MATERIAL.

The following pure bred lines have been used as starting material.

- | | | |
|----|----------------------------|---|
| 1. | <i>Flower colour:</i> Blue | <i>Seed colour:</i> Earth-brown, marbled and white punctured. The ordinary seed colour. |
| 2. | » : » | » : White punctured and earth-brown, not marbled. This is the variety »Weibull's Meteor». |
| 3. | » : Tinged blue | » : Earth-brown, marbled and white punctured. |
| 4. | » : Bluish red | » : Earth-brown, marbled and white punctured. |
| 5. | » : Violet | » : Rust-brown, marbled and white punctured. |
| 6. | » : White | » : White. |

In the course of the experiments the following new types have been obtained as recombination products:

- | | | |
|----|------------------------------|--|
| 1. | <i>Flower colour:</i> Violet | <i>Seed colour:</i> Rust-brown and white punctured, not marbled. |
| 2. | » : Pure red | » : Rust-brown, marbled and white punctured. |
| 3. | » : Tinged red | » : Earth-brown, marbled and white punctured. |

The colour of the flower changes with age in this lupine as well as in other species of the genus. The different types are, however, very distinct, and no difficulty is met with in classifying the types. While in all coloured types coloration appears in the vegetative organs, as in the cotyledons, in the petioles, in the bracts and sepals, no such coloration is found in the white flowered types, where the enumerated parts are pure green.

DETAILS OF THE TYPES.

THE FLOWER COLOUR.

The blue type (Pl. II, fig. 1). Standard blue, wings blue with the upper edge of the upper third bluish violet and with a bluish white lower part, strongly marked with deep blue coloured nerves. Also the lower edge of the lower part lighter. Keel white with blue nerves and with an intensive dark blue beak. The two upper sepals of the calyx quite blue, the two lateral, smaller ones also almost blue coloured, the median, strongly developed sepal green with the exception of blue coloured tip and nerves. The membranaceous bract with blue spots and striae concentrated in the margin of the leaf. This makes the unopened inflorescence quite blue. The colour of the axis of the inflorescence in the upper part strongly blue, disappearing downwards. The colour of the flower more intensive with age.

The tinged blue type (Pl. II, fig. 5). Standard of flowers just opening almost white on the inside with only a faint tinge of blue; the outside, the tip and the flanks with a weak but distinct blue colour. The median and basal parts white. The upper edge of the wings white, the lower edge with a faint blue colour, disappearing from the place of union downwards. Keel white with no trace of colour (the beak of the keel of the blue type deep blue coloured). The yellow anthers visible through the keel.

The blue coloured parts become larger with age, and the coloration becomes more intensive. At last a violet tinge appears on the inside of the standard and on the upper and outer parts of the wings. However, the colour of the oldest flowers of this type is not anything like the colour of the youngest flowers of the blue type. The keel remains uncoloured. The colour of the bracts and sepals as in the blue type, the colour of the unopened inflorescence, consequently, blue. The colour of the axis of the inflorescence also blue.

The violet type (Pl. II, fig. 2). Standard violet with blue nerves,

with a bluish tinge at the base. Wings violet with blue nerves, the lower edge from the place of union downwards with a bluish tinge; the upper edge lighter, in its upper part, above the place of union, reddish violet. Keel white with violet nerves and blackish violet beak. Blue coloured parts enlarging with age, the colour intensifying. Sepals and bracts violet, the distribution of colour as in the former type. The colour of the axis brownish.

The bluish red type (Pl. II, fig. 3). Standard red with blue nerves. Wings red with blue nerves, the colour weaker in the back parts of the upper edge, and in the basal parts on the whole. The area above the union of the wings either quite blue or sharply defined by a blue line. Keel white with blackish red beak. Older flowers with a diffuse blue colour, intensifying with age. Sepals and bracts reddish with a tinge of blue. The distribution of the colour in these organs as in the blue type. The axis bluish red.

Bluish red is ordinary called red in *Lupinus angustifolius*. Bluish red is probably the colour called rose by FRUWIRTH and red by VESTERGAARD, as mentioned in the above.

The pure red type (Pl. II, fig. 4). I have intentionally adopted this name to mark it off from the bluish red type with which it is easily confused. As regards the red colour no difference is seen in the both types, but every trace of blue colour in the flower and in other parts is absent from the pure red. The axis is brownish red. The difference between the two colour types is quite distinct and errors in classifying them are out of question to the trained observer; a less trained eye, however, may have some difficulty in separating the types. This difference is also noticeable in the seed colour. Cp. the description of the seed colour, pag. 304, and the characteristic of the seed colour in immature stages, pag. 331.

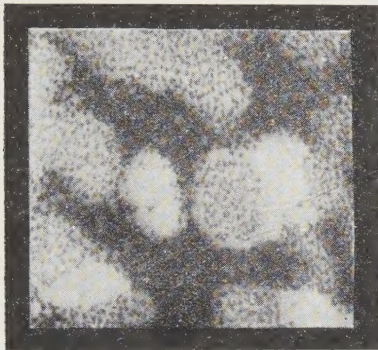
The tinged red type (Pl. II, fig. 6). Standard and wings with a faint, yellowish tinge of rose. Keel as in the tinged blue type, without colour, even in the beak. Sepals, bracts and axis as in the bluish red type.

The white type. Standard and wings in early stages pure white, coloured with age. Intensity of the colour varies with different environmental conditions; it is sometimes so marked that a flower in the proper stage of development hardly deserve to be called white coloured. An old individual of a white line seems to be stronger coloured than the »dilution forms» tinged blue and, particularly, tinged red, since the colour of these is diffuse, while the colour of the whites is concentrated in the wings.

There is one important point of difference, however, between the flowers of the whites and those of the tinged types, viz. the coloration of the latter ones even in early stages. The young flowers of the whites show no trace of colour; the sepals, bracts and axis are quite green.

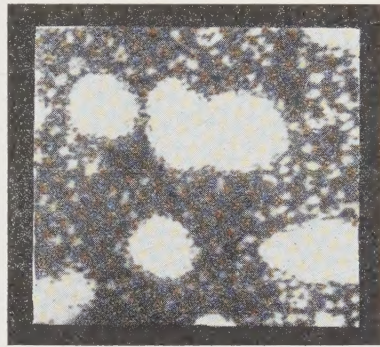
THE SEED COLOUR.

The seed colour of *Lupinus angustifolius* depends on the presence of pigment in the palisades of the seed coat, according to the investigations of KAJANUS and FRUWIRTH. The figure reproduced in the work of FRUWIRTH (1915, pag. 218) shows that the pigment layer is defined to the centre of the palisade cells. The ordinary colour of the



a

Marbled; white flecks, dark areas composed of closely packed pigment grains, the lighter areas with scattered grains.



b

Non-marbled; white flecks, dark areas as in *a*, the lighter areas lacking.

Fig. 1.

seed is this (Pl. II, fig. 8). On a lighter or darker coloured pearl-gray ground there is found an earth-brown marbling of varying limits together with white flecks sharply defined. When examined under a magnifying lens the characteristics of this coloration will be found (see fig. 1). The pigment grains are collected in small groups; the heaping up of these groups or the scattered occurrence of the groups give rise to the above mentioned characteristic colour schemes as shown in the figure. They are very closely heaped up in the dark marbled areas, they are more scattered in the pearl-gray fields, and in the white parts only sporadic dots are found. The limits between the different coloured areas are remarkably sharp. I have characterized this type as the earth-brown, marbled. KAJANUS mentions three

varieties of this type differing in the intensity and in the distribution of the colour; only the darker one of his types, which at the same time is the one generally found, has been used in my experiments, but seeds resembling the lighter, thin coloured types have often been found as modifications.

The fourth type proposed by KAJANUS has been used in one of my experiments (Pl. II, fig. 7). It lacks the pearl-gray ground-colour; in its place the earth-brown marbling colour covers the whole surface of the seed with the exception of the white flecks. The pigment groups are found here only in two grades of density, sporadically in the white flecks and closely compacted in the earth-brown areas. The intermediate grades of density do not occur, and the gray colour, consequently, does not occur either (fig. 1 b). I have characterized this type as the earth-brown, not marbled. It is very easy to distinguish this type from the marbled as no transitions are found.

The earth-brown seed colour is found correlated with blue, tinged blue, bluish red and tinged red flowers, but is not found in violet, pure red and white.

The rust-brown seed colour, (Pl. II, fig. 9 and 10) due to another kind of pigment, is not mentioned in the literature. This type includes also marbled and non-marbled varieties. The marbled variety (Pl. II, fig. 10) has a gray field, almost cream-coloured, a rust-brown marbling and white flecks. Seeds from the earth-brown type are sometimes modificatory rust-brown when poorly developed. If only sound and fully developed seeds are taken for comparison, no difficulty is met with in classifying the types. The rust-brown seed colour is found correlated with violet and pure red flowers.

The non-marbled variety of the rust-brown type has been obtained as a recombination product from my experiments. It also lacks the gray ground colour as does its earth-brown analogue. It may appear peculiar that the gray ground colour and the marbling colour of the three-coloured type depend on different quantities of the same pigment. The investigations of FRUWIRTH and KAJANUS as well as my own experiments bear out this fact, however, and it is further strengthened by the observations made on the colour of seeds still immature and enclosed in the green pods. The gray as well as the marbled parts of the seed have the same colour at this stage, and the difference is wholly one of intensity. In the earth-brown type there are two different grades of intensity of blue, in the rust-brown type of violet.

The white seed (Pl. II, fig. 11) has a faint rust-brown, hook-for-

med scar at the hilum; the whole surface of the seed is generally found to have a touch of the same marbling colour. Cp. the coloration of the white flowers when old.

I have only found white seeds in lines with white flowers.

As to the methods employed it should be stated that isolation by means of parchment bags has been used in all cases (with the exception of Nos. 228—236—20). Consequently, the families are all progenies from isolated mother plants. It seems, however, as were self-fertilization the exclusive method of fertilization in *L. angustifolius*. The families just mentioned (94 plants in all) showed no case of vicinism when allowed to seed whithout bagging, and the same has been found in other families with recessive flower colours not mentioned in the tables, viz. 3 tinged blue, 2 tinged red and 1 pure red families, 105 individuals in all.

No case of vicinism has been found in these 199 cases in spite of the most favourable opportunities of hybridization. Further investigations on this point are in progress. The same has been pointed out by VESTERGAARD, who did not use any isolation in his experiments.

The following crosses have been made between the types discussed in the above.

TABLE OF CROSSES.

Cross	1: White flower colour » seed colour	} × {	Blue flower colour Earth-brown, marbled seeds
»	2: Violet flower colour Rust-brown, marbled seeds	} × {	Blue flower colour Earth-brown, non- marbled seeds
»	3: Bluish red flower colour Earth-brown, marbled seeds	} × {	Blue flower colour Seed colour as male parent
»	4: Tinged blue flower colour Earth-brown, marbled seeds	} × {	Blue flower colour Seed colour as male parent
»	5: White flower colour » seed colour	} × {	Tinged blue flower colour Earth-brown, marbled seeds
»	6: Violet flower colour Rust-br. marbled seed colour	} × {	White flower colour » seed colour

Cross 7:	Bluish red flower colour Earth-brown, marbled seeds	$\left\{ \begin{array}{l} \times \end{array} \right\}$	$\left\{ \begin{array}{l} \text{White flower colour} \\ \text{» seed colour} \end{array} \right.$
8:	Tinged blue flower colour Earth-brown, marbled seeds	$\left\{ \begin{array}{l} \times \end{array} \right\}$	$\left\{ \begin{array}{l} \text{Violet flower colour} \\ \text{Rust-brown, marbled} \\ \text{seeds} \end{array} \right.$
9:	Bluish red flower colour Earth-brown, marbled seeds	$\left\{ \begin{array}{l} \times \end{array} \right\}$	$\left\{ \begin{array}{l} \text{Violet flower colour} \\ \text{Rust-brown, marbled} \\ \text{seeds} \end{array} \right.$
10:	Bluish red flower colour Earth-brown, marbled seeds	$\left\{ \begin{array}{l} \times \end{array} \right\}$	$\left\{ \begin{array}{l} \text{Tinged blue flower} \\ \text{colour} \\ \text{Seed colour as male} \\ \text{parent} \end{array} \right.$

GENETICAL FORMULAE.

Before discussing the segregations it seems desirable to give a summary of the genetical formulae. The formulae shall be fully discussed later.

R: a factor for pure red flower colour and rust-brown seed colour; it is, in addition, the fundamental colour factor; the *rr*-types have white flowers and white seeds.

B: a bluing factor giving bluish red flowers and earth-brown seeds with *R*.

V: transforms the pure red colour into violet, necessitating the presence of *R*; *V* does not influence the seed colour.

The factors *B* and *V* give the full blue colour when both present; in the absence of *R* no coloration is attained by any of them. Thus the formula *RBV* is required for blue colour.

F: a factor necessary for the complete development of the colour. The colour produced by the other factors become diluted when *F* is absent. Blue becomes tinged blue, bluish red becomes tinged red. The diluted forms of violet and pure red have not yet been found. The seed colour is not influenced by this factor.

M: the marbling factor. The pigment in the seed coat becomes closely compacted in some parts and thinner in other when *M* is present. In the absence of *M* no marbling occurs the pigment being distributed evenly on the surface, interrupted only by white flecks.

The different constant phenotypes apart from the marbling are thus constituted as follows:

Blue	flower colour, earth-brown seed colour	=	<i>RRBBVVFF</i>
Tinged blue	» » »	=	<i>RRBBVvff</i>
Bluish red	» » »	=	<i>RRBBvvFF</i>
Tinged red	» » »	=	<i>RRBBvvff</i>
Violet	» rust-brown »	=	<i>RRbbVVFF</i>
(?	» » ? »	=	<i>RRbbVVff</i>) not yet obtained.
Pure red	» » »	=	<i>RRbbvvFF</i>
(?	» » ? »	=	<i>RRbbvvff</i>) not yet obtained.
White	» white »	=	<i>rrBBVVFF</i>
»	» » »	=	<i>rrbbVVFF</i> etc.

THE RESULTS OF THE SEGREGATIONS.

The detailed results of the segregations and the numerical relations within the different families are tabulated in tables 7—26, pp. 345—362. The numbers of individuals of the different families are rather small, as seen, and therefore it is not possible to decide upon the question whether within the families belonging to the same type of segregation small but significant differences are present, as for instance in the degree of linkage etc.; only the totals of the families with the same mode of segregation have been considered here. When treating the proportions between the F_3 -families of different types of segregation only the relation between the constant dominants and the heterozygous families have been determined; the constant recessives have not been considered. Thus the proportions expected have been referred to the number 3 ($1 + 2$) instead of 4 ($1 + 2 + 1$) in the case of simple segregation. In the case of di-hybrid segregations the proportions have not been calculated from the constant 16, but each family from double dominant and simple recessive mother plants have been calculated from the numbers $1 + 2 + 2 + 4$ and $1 + 2$ respectively. It should also be mentioned that F_3 -families with less than 20 individuals have not been considered.

It is to be expected, according to the genetical formulae, that all crosses between blue colour on the one side and tinged blue, bluish red, violet and the whites constituted *rrBBVVFF* on the other should give simple segregation, as each of these latter ones differ genotypically from the blue ones only by the absence of one factor. The same type of segregation should result, and for the same reason, when the earth-

brown seed of the blue flowers are crossed with the rust-brown in the violet and with the white seed of the whites. All these combinations have been made (crosses 1, 2, 3, 4), and both the F_2 - and the F_3 -generations have given the results expected.

Cross 1 (table 7).

White flower colour	} × {	blue flower colour
» seed colour		earth-brown seed colour
<i>rrBBVVFF</i>		<i>RRBBVVFF</i>

F_1 is blue and earth-brown, and the segregation in

F_2 : 289 blue : 73 white, expected 271,5 : 90,5.

The difference = 17,5 is 2,1 times the standard error (8,24) and, consequently, somewhat great, yet it is not so large as to lie beyond the limits prescribed. F_3 was spoiled. The inheritance of the seed colour was not followed in this cross.

Cross 2 (tables 8—9).

Violet flower colour, rust-brown seed colour (*RRbbVVFF*) × blue flower colour, earth-brown seed colour (*RRBBVVFF*).

F_1 is blue with earth-brown seeds, and F_2 has given:

215 blue : 73 violet (table 8)

expected: 216 72 D/M = 1,0/7,35 = 0,14.

3 violet F_2 -plants have given constant violet progenies in F_3 (table 9) with 247 individuals in all. Among 9 F_3 -families from blue parent plants 1 has given a constant blue progeny (22 individuals), and 8 have segregated in:

456 blue : 154 violet

expected: 457,5 : 152,5 D/M = 1,5/10,69 = 0,14.

The results of the segregation in F_2 as well as in F_3 are, as seen, pretty satisfactory, although the proportions between the constant and the segregating F_3 -families might have been better. Instead of the expected 3 : 6 proportions 1 : 8 was obtained. The low number of plants makes the deviation allowable. The seed colour was determined in F_2 in 209 blue and 69 violets; all blue were found to have earth-brown seeds, all the violets rust-brown seeds.

Cross 3 (tables 10—11).

Bluish red × blue flower colour, no difference in seed colour.
RRBBvvFF *RRBBVVFF*.

F_1 is blue, and F_2 segregates in

122 blue : 40 bluish red (table 10)

expected: 121,5 : 40,5 $D/M = 0,5/5,51 = 0,09$.

The following segregation numbers were obtained in F_3 :

296 blue : 101 bluish red

expected: 297,75 : 99,25 $D/M = 1,75/8,63 = 0,20$.

The relation between the number of constant and segregating families is 6 : 8, expected: 4,67 : 9,33. The constant blue families have 296 individuals in all. From 6 bluish red parent plants 202 individuals of the same colour were obtained. Thus the results are pretty well in accordance with the theoretical expectation.

Cross 4 (tables 12—13).

Tinged blue $\times$ blue flower colour, no difference in seed colour.

RRBBVVff RRBBVVFF

F_1 , having the formula *RRBBVVff* and heterozygotic in factor F only, is blue, but upon closer study it is found that a weakening of the colour has taken place. The influence of the F -factor is thus seen to be somewhat stronger in the duplex stage than in the simplex stage. The segregation numbers in F_2 as well as in F_3 are very good:

F_2 396 blue : 138 tinged blue (table 12)

expected: 400,5 : 133,5 $D/M = 4,5/10,0 = 0,45$

F_3 324 blue : 107 tinged blue (table 13)

expected: 323,25 : 107,75 $D/M = 0,75/8,99 = 0,08$.

The relation between the constant and the segregating F_3 -families, 6 : 11, comes in addition as close as possible to the theoretical expectation, and the progenies of all tinged blue coloured plants investigated have been found to be constant in accordance with the theory.

Simple Mendelian segregation is to be expected in some other combinations according to the theory. No primary crosses have been made in these cases, but the F_3 of some di-hybrid crosses includes families from parent plants with the same kind of heterozygosis as that of the primary crosses if made. The proportions to be expected, 3 : 1, are found in all cases. The following cases should be observed:

Tinged blue $\times$ tinged red in F_3 of cross 10 (table 26),

Bluish red $\times$ pure red in F_3 of cross 9 (tables 23—24),

Violet $\times$ pure red in F_3 of cross 9 (table 24),

Bluish red $\times$ tinged red in F_3 of cross 10 (table 26),

Bluish red $\times$ white ($rrBBvvFF$) in F_3 of cross 7 (table 19),
 Tinged blue $\times$ white ($rrBBVVff$) in F_3 of cross 5 (table 15),
 Violet $\times$ white ($rrbbVVFF$) in F_3 of cross 6 (table 17).

With the exception of the combinations tinged red $\times$ white, with the constitution $rrBBvvff$, and pure red $\times$ white, with the constitution $rrbbvvFF$, all those combinations have been investigated, where simple segregation is to be expected, and the segregation has in all these cases followed normal and good proportions without any selection or other disturbances.

Three crosses have been made between whites on the one side and coloured but not blue types on the other. All these combinations gave a synthesis of blue and segregated in F_2 in the ratios 9 : 3 : 4. The proportions found in F_3 substantiate the results and correspond with the hypothesis as well. These are the crosses 5, 6 and 7.

Cross 5 (tables 14—15).

White seed- and flower colours ($rrBBVVFF$) $\times$ tinged blue flower colour ($RRBBVVff$); earth-brown, marbled seed.

F_1 has slightly diluted blue flower colour and earth-brown seeds. The simplex stage of the factor F accounts for the slight dilution of the colour (Cp. cross 4).

F_2 53 blue : 10 tinged blue : 18 white (table 14)
 expected: 45,₅₆ : 15,₁₉ : 20,₂₅

$$D/M \frac{7,44}{4,66} = 1,60 \qquad \frac{5,19}{3,51} = 1,45 \qquad \frac{2,25}{3,90} = 0,58$$

F_3 of the same cross gave:

 337 blue : 127 tinged blue : 138 white (table 15)
 expected: 338,₆₂ : 112,₈₈ : 150,₅

$$D/M \frac{1,62}{12,17} = 0,13 \qquad \frac{14,02}{9,58} = 1,47 \qquad \frac{12,5}{10,62} = 1,18$$

It is clear that the segregation of this cross takes place in the ratio 9 : 3 : 4, even if the deviations often lie beyond the limits of the standard error in each generation. When the results in F_2 and F_3 are summed up, however, the differences become smoothed out, and the results conform to the theory, the quotients D/M being 0,₄₅; 0,₈₈; and 1,₁₇ respectively. Also the rest of the results in F_3 corresponds with the hypothesis. The observed numerical relations are pretty well in accordance with the calculated as seen in the following.

158 blue : 48 tinged blue

$$\text{expected: } 154,5 : 51,5 \quad D/M = \frac{3,5}{6,22} = 0,56$$

224 blue : 78 white

$$\text{expected: } 226,5 : 75,5 \quad D/M = \frac{2,5}{7,53} = 0,33$$

179 tinged blue : 56 white

$$\text{expected: } 176,25 : 58,75 \quad D/M = \frac{2,75}{6,64} = 0,41$$

The constant and the segregating families occur in a number, which is very near to the theoretical expectation. From 32 blue parent plants the result was:

7 constant blue : 13 9:3:4-segregates : 6 blue and tinged blue : 6 blue and white families

$$\begin{array}{ccc} \text{expected: } 3,56 & 14,22 & \overbrace{7,11 \quad 7,11} \\ D/M \quad \frac{3,44}{1,78} = 1,93 & \frac{1,22}{2,82} = 0,43 & \frac{1,11}{1,94} = 0,58 \end{array}$$

10 tinged blue F_2 -plants gave in F_3 :

5 constant tinged blue : 5 tinged blue and white families

$$\text{expected: } 3,33 : 6,67 \quad D/M = \frac{1,67}{1,57} = 1,06$$

As seen the mode of segregation in this cross is typical of a synthetic cross. It should be noticed, however, that the synthesis does not originate through the coming together of a fundamental colour factor and the blue colour factors. This combination is realized already in the tinged blue parent plant. It is the meeting of the fundamental factor with the intensity factor F that makes the synthesis.

45 blue, 8 tinged blue and 5 white F_2 -plants have been examined as to seed colour; all the blue and the tinged blue had earth-brown seeds, and all the whites had white seeds.

Cross 6 (tables 16—17).

Violet flower colour, earth-brown seeds $\times$ white flowers and seeds.

$RRbbVVFF$

$rrBBVVFF$

F_1 had blue flowers and earth-brown seeds. In F_2 we expect, according to the theory, the ratios 9 : 3 : 4. We find:

27 blue : 16 violet : 8 white (table 16)

$$\begin{array}{l} \text{expected: } 28_{,09} \qquad 9_{,56} \qquad 12_{,75} \\ \text{D/M } \frac{1_{,69}}{3_{,54}} = 0_{,48} \quad \frac{6_{,44}}{2_{,79}} = 2_{,31} \quad \frac{4_{,75}}{3_{,09}} = 1_{,53} \end{array}$$

The number of individuals in the whites and in the violets seems to indicate quite other ratios than 9 : 3 : 4. The results obtained in F_3 show, however, that this ratio must be correct, and that the abnormal number in F_2 is a case of an unusual great deviation, although within the range allowed by the theory. From blue parent plants partly di-hybrids have been obtained showing the following numerical relation:

216 blue : 76 violet : 92 white (table 17)

$$\begin{array}{l} \text{expected: } 216 \qquad 72 \qquad 96 \\ \text{D/M } \qquad 0 \qquad \frac{4}{7_{,65}} = 0_{,52} \quad \frac{4}{8_{,49}} = 0_{,47} \end{array}$$

partly mono-hybrids with the following numerical relations:

135 blue : 50 violet

$$\text{expected: } 138_{,75} \quad 46_{,25} \quad \text{D/M } \frac{3_{,75}}{5_{,90}} = 0_{,64}$$

169 blue : 43 white

$$\text{expected: } 159 \quad 53 \quad \text{D/M } \frac{10_{,0}}{6_{,30}} = 1_{,59}$$

No constant blue families have been found, however. This is probably due to the small number of families. 13 families from blue mother plants, distributed in the following classes have been followed:

0 constant blue : 5 9:3:4-segregates : 4 blue and violet : 4 blue and white

$$\text{expected: } 1_{,44} \qquad 5_{,74} \qquad 2_{,89} \qquad 2_{,89}$$

3 constant violets and 5 3:1-segregating families have been obtained from violet plants. The proportion expected is 2_{,67} : 5_{,33} and the total number of individuals in the segregating families was:

242 violet : 93 white

$$\text{expected: } 251_{,25} \quad : 83_{,75} \quad \text{D/M } \frac{9_{,25}}{7_{,93}} = 1_{,17}$$

One white plant gave a constant white progeny.

The seed colour in the F_2 -plants has been examined in 26 blue plants, 14 violets and 6 whites. The colour of the seeds in the blue

plants was earth-brown, in the violets rust-brown and in the whites white.

Cross 7 (tables 18—19).

Bluish red flower colour, earth-brown seeds (*RRBBvvFF*) $\times$ white flowers and seeds (*rrBBVVFF*).

F_1 gave blue flowers and earth-brown seeds

F_2 41 blue : 15 bluish red : 17 white (table 19)

expected: 41,₀₆ 13,₆₉ 18,₂₅

$$D/M \quad \frac{0,06}{4,24} = 0,01 \quad \frac{1,31}{3,34} = 0,39 \quad \frac{1,25}{3,70} = 0,34$$

The numerical relations are good also in the F_3 -generation:

F_3 100 blue : 30 bluish red : 42 white (table 20)

expected: 96,₇₅ 32,₂₅ 43

$$D/M \quad \frac{3,25}{6,51} = 0,50 \quad \frac{2,25}{5,12} = 0,44 \quad \frac{1,00}{5,68} = 0,18$$

The mono-hybrid F_3 -numbers are satisfactory even if not quite as good as the above; they are:

33 blue : 14 bluish red

expected: 35,₂₅ 11,₇₅ $D/M = \frac{2,25}{3} = 0,75$

47 blue : 24 white

expected: 53,₂₅ 17,₇₅ $D/M = \frac{6,25}{3,65} = 1,71$

33 bluish red : 7 white

expected: 30 10 $D/M = \frac{3}{3,22} = 0,93$

Blue coloured F_2 -plants have given the following F_3 -families:

2 constant blue : 5 9:3:4-segregates : 2 blue and bluish red : 2 blue and white

expected: 1,₂₂ 4,₈₈ 2,₄₄ 2,₄₄

5 bluish red F_2 -plants gave 4 constant bluish red and one 3:1-segregating family. The numbers to be expected are 1,₆₆ constant and 3,₃₄ segregating families. The class distribution in F_3 shows, as seen, very poor correspondence with the expectation; the inversion of the values would give almost the ideal relations. The possibility of such an inversion is not improbable when the small numbers of families are considered. 7 white flowering plants gave as many constant white families.

As to the seed colour the above mentioned whites and their progeny had all white seeds, the rest of the F_2 -plants examined with regard to seed colour, 25 in all, had earth-brown seeds. 20 of these were blue coloured, the other 5 tinged blue.

The di-hybrid crosses just discussed, where *one* of the parents has been white follow the hypothesis exactly, and the results of the segregations are quite satisfactory. The group of di-hybrid crosses where both parents have been coloured, although not blue coloured, has now to be discussed (crosses 8, 9 and 10).

Cross 8 (tables 20—21).

Tinged blue flowers, earth-brown seeds ($RRBBVvff$) $\times$ violet flowers, rust-brown seeds ($RRbbVVFF$).

F_1 slightly diluted blue, earth-brown seeds.

F_2 should segregate in blue, violet, tinged blue and tinged violet in the ratios 9:3:3:1, according to the hypothesis. The result was:

470 blue : 241 violet : 255 tinged blue (table 14).

Thus the expected mode of segregation has not been realized. A double recessive of the violet tinged type is lacking; mere chance is excluded on account of the great number of individuals. About 60 plants of this type are to be expected; however, since none has been obtained, a deviation 8 times the standard error has to be assumed, a deviation so large as to lie far beyond the allowed range. It could be argued, perhaps, that the absence of the F -factor did not influence the violet colour. This seems very improbable, as the number of the blue and tinged blue groups is abnormal, the former is too small, the latter too large to account for the ratios 9 blue : 3 tinged blue : 4 violet to be expected in such a case; the results obtained in F_3 also invalidate this argumentation. If valid, mono-hybrid families together with di-hybrids should be obtained from blue coloured F_2 -plants, and mono-hybrid families in addition to the constant families should originate from tinged blue parent plants. This is not the case, however. The numerical relations showing the best correspondence with the numbers found are 2 blue : 1 violet : 1 tinged blue giving when applied to the number of individuals in question:

483 blue : 241,5 violet : 241,5 tinged blue

$$D/M \quad \frac{13}{15,5} = 0,84 \quad \frac{0,5}{13,5} = 0,04 \quad \frac{13,5}{13,5} = 1,0$$

F_3 repeats this di-hybrid segregation with:

	1160 blue	: 586 violet	: 605 tinged blue
expect:	1175, ₅₀	587, ₇₅	587, ₇₅
D/M	$\frac{15,50}{24,2} = 0,64$	$\frac{1,75}{21} = 0,08$	$\frac{17,25}{21} = 0,77$

There are additional deviations from normal segregation to note. Constant blue flowered families, as well as 3:1-segregating families from blue parent plants, are wholly absent. All families from such parent plants give the di-hybrid type of segregation in the ratios 1:2:1. All families from violet and tinged blue parent plants are, furthermore, constant and do not show any segregation. All these deviations in the proportions of the families and of the phenotypes are characteristic of an absolute or close linkage.

It is dilution and the violet colour which, phenotypically seen, never or rarely enter into combination. Genotypically it is explained by assuming a repulsion between the factors B and F ; the phenotypical correlation between violet and fully developed colour should, in other words, depend on the closeness between the factors representing bluish red and full colour according to the chromosome-theory. This depends of course on the construction of the factorial scheme. The violet colour caused by the factor V appears only when the B -factor has been excluded, and vice versa. Consequently, this inverse proportionality between the phenotypical manifestation and the genotypical mode of characterization will be found in all the following crosses, where linkage occurs. This would have been avoided had the CASTLE-MORGAN method of factor denotation been adopted (cp. MORGAN 1915, pag. 253). The genetical formulae would have been constructed in the following way: Blue = wild type; bluish red is caused by mutation of the B -factor, denoted b ; we denote further violet colour by v instead of bV , and pure red by bv und not by bvR ; tinged colour is caused by the absence of F ($=f$) as before. The repulsion in cross 8 would then be caused by the close linkage between the factors f and v . The fact that our case corresponds in colours and in the mode of segregation with analyses previously made favoured, however, the adoption of the traditional factor denotation.

The degree of linkage can not be determined, as no recessives appear. It may be absolute, but partial linkage of a relatively high degree is also conceivable. The results obtained allow but an approximate estimate of the minimal value of its strength. The total number of individuals of the di-hybrid segregation in F_2 and F_3 is 3317.

Assuming a relative frequency between recombinated gametes and non-recombinated ones (the former category not yet found in this case) of $1 : 10 : 10 : 1$ ($BF : Bf : bF : bf$) a number of 6,⁸⁵ double recessives would originate, and the probability of their non-appearance is about $1 : 1000$. Thus it is very improbable that the non-recombinated gametes are less than 10 times the recombinated ones. They are rather much more numerous. This belief is strengthened when the numerical relations of the F_3 -families are considered.

The F_3 -method for determining gametic ratios will be further treated in the following, where a case of partial linkage is discussed (pag. 323). As it is shown here the gametic ratio may be determined by the proportions between F_3 -families of two different groups belonging to different types of segregation.

A number of 60 segregating families of repulsion type, 22 constant violets and 44 constant tinged blue have been obtained in the present cross. They all belong to *one* of the family-groups just mentioned, the other group, where the families segregating in the ratios $3 : 1$ form the bulk, is altogether absent. As in the » F_2 -analysis» the minimal value of the strength of the linkage may thus be determined and it is found that the F_3 -method gives much better result. The minimum ratio $1 : 10$ just calculated is, according to the F_3 -analysis, out of question; the ratio $1 : 36$ may be assumed with just the same probability to be the minimum. The gametic representation would necessitate 7 families out of the 126 grown belonging to the absent group, and the probability that all these are excluded is about $1 : 1000$. It is therefore rather safe to assume a higher frequency of the non-recombinated gametes. The probability of the exclusion of the said families is about $1 : 25$ at a gametic frequency of $1 : 100$; the probability of their exclusion and their presence are equal first at a gametic frequency of $1 : 300$, when the total number of families is 126. These calculations show clearly the advantage of using the F_3 -method in cases of repulsion. It has been found to give much greater precision than the analysis of the F_2 , even when the number of the families has been relatively small. It is clear that the present case is a case of close linkage, and it is of great interest to continue the cross.

The result of the examination of the seed colour in this cross gave: 40 violets with rust-brown seeds, 87 blue and 59 tinged blue with earth-brown seeds.

Cross 9 (tables 22—24).

Bluish red flowers, earth-brown seeds (*RRBBvvFF*) $\times$ Violet flowers, rust-brown seeds (*RRbbVVFF*).

*F*₁ Blue flowers, earth-brown seeds.

The deviations from expectation in *F*₂ are great also in this cross. All the expected phenotypes are present, it is true, and pure red is obtained for the first time, but the ratio 9 : 3 : 3 : 1 is greatly deformed. It is clear that the deviation is due to disturbances in the gametic representation in the shape of repulsion. It should be mentioned that

TABLE 1.

Reference number	Generation	Frequency of phenotypes					Ass. coeff. of observed phenotypic ratio	Standard error M	Ass. coeff. of calcul. phenotypic ratio	Gametic ratio	Crossover percentage
		<i>BV</i>	<i>Bv</i>	<i>bV</i>	<i>bv</i>	Total					
		1	2	3	4	5	6	7	8	9	10
<i>Repulsion.</i>											
1	<i>F</i> ₂	556	269	226	15	1066	0.7587	± 0.0587	0.7567	1 : 3.1 : 3.1 : 1	24.4
2	<i>F</i> ₃	1142	540	542	20	2244	0.8290	± 0.0153	0.8305	1 : 3.9 : 3.9 : 1	20.4
3	<i>F</i> ₄	9	8	3	0	20	—	—	—	—	—
4	<i>F</i> ₂ + <i>F</i> ₃ + <i>F</i> ₄	1707	817	771	35	3330	0.8248	± 0.0285	0.8233	1 : 3.8 : 3.8 : 1	20.8
<i>Coupling.</i>											
5	<i>F</i> ₃	241	28	30	62	361	0.8935	± 0.0301	0.8935	4.7 : 1 : 1 : 4.7	17.6
6	<i>F</i> ₄	204	28	32	33	297	0.7651	± 0.0663	0.7656	3 : 1 : 1 : 3	25.0
7	<i>F</i> ₃ + <i>F</i> ₄	445	56	62	95	658	0.8482	± 0.0303	0.8510	3.9 : 1 : 1 : 3.9	20.4

there is a remarkably great number of recessives in the family 597—18 (table 22), and the segregation agrees with the proportions 9 : 3 : 3 : 1. The number of individuals, however, is not large enough to substantiate the view that the type of segregation differs from the type characteristic of the rest of the families, and this is not either to be expected. Unfortunately no seed could be obtained from this family, and therefore no *F*₃-generation could be raised.

From the result of the segregation it is seen at once that the gametic frequency is about 1 : 3 or 1 : 4. In order to arrive at an accurate determination of the ratio the method of COLLINS and BRIDGES (COLLINS 1912, BRIDGES 1914) was adopted. The association coefficient (YULE'S ASS.) of the segregating ratios obtained has thus been calcula-

ted (col. 6, table 1) and compared with a series of ass. coefficients of several ideal phenotypical ratios belonging to various gametic ratios. The results are put together in table 1 (pag. 317).

The accuracy of the values obtained in this or similar ways, as EMERSON (1916) has pointed out, depends greatly on the question whether or not the proportions between certain groups of the zygotic distribution series meet the demands which have to be raised (apart from the characteristic distribution at the gametic frequency in question). The characteristic feature of di-hybrid segregation values with $9:3:3:1$ as ground-type, whether free combination or linkage of the coupling or repulsion type are exhibited, is the equality of the middle groups ($Bv = bV$, or $\frac{Bv + bV}{2}$). The first group, BV , must be

three times the last one when the sum of the middle groups ($Bv + bV$) is subtracted from the first group. That is, $BV - (Bv + bV) = 3 bv$,

or $\frac{BV - (Bv + bV)}{3} = bv$. These points are demonstrated in the

scheme, pag. 320. If different linkage values are obtained for the same characters the accuracy of the different values should be controlled from this point of view. Such a control of the values in this cross shows that in two cases remarkable deviations from expectation have resulted, viz. in the case of number 1 of the F_2 , table 1, and in ref. number 6 in F_4 in coupling. In the first case the difference between the middle groups is rather great. It is seen, however, that this deviation is of no importance as the value of the association coefficient, calculated with the middle groups reduced to half of the sum of values actually obtained, changes in so small a degree that it still shows the best correspondence with the gametic ratio $1:3,1$. Thus the deviation from other calculations showed by this ratio does not depend on the difference in the values of the middle groups.

In the second example, viz. the coupling in F_4 (ref. number 6), the last group, bv , is found to be too small in relation to $BV - (Bv + bV)$. The values obtained are 33 and 144 respectively. The ideal ratio would be $44,25:132,75$, consequently a deficit of more than 11 recessives. If the association coefficient is calculated on the ideal ratio just mentioned the gametic frequency becomes $3,3:1$ instead of $3:1$, and the linkage value becomes $23,3$ instead of 25. It is therefore to be concluded from the mere disproportion between the first and the last groups that the result with regard to the coupling in F_4 (ref. number 6) is doubtful.

TABLE 2.

Ass. coeff. of the phenotypic ratios corresponding to various gametic ratios.

Gametic ratio	Coupling	Repulsion
1,5 : 1	0,3494	0,3425
2,0 : 1	0,5575	0,5422
2,5 : 1	0,6845	0,6646
3,0 : 1	0,7656	0,7445
3,5 : 1	0,8198	0,7986
4,0 : 1	0,8575	0,8373
4,5 : 1	0,8847	0,8659
5,0 : 1	0,9049	0,8875
5,5 : 1	0,9114	0,9043

The linkage values obtained may be further tested with the help of the standard error of the ass. coeff. determined according to YULE (1900) and listed in table 1, col. 7, and with the help of the table of the association coefficients for a series of gametic ratios (table 2). Both coupling and repulsion values answering to about 3 : 1 and 1 : 3 respectively are obtained if the threefold standard error is subtracted from the association coefficient of the observed values. This value marks the lower limit, and it is very improbable that the numerical values correspond to a gametic ratio lower than this. The corresponding upper limit lies higher. The ass. coeff. of repulsion reaches a value differing from the ass. coeff. by three times the standard error first at the gametic ratio 1 : 5,5, and the limit lies still higher in question of coupling values. When the simple standard error is used, the corresponding limits are 1 : 3,5 and 1 : 4,3.

Thus it is seen that the standard errors allow great fluctuations of the linkage values, and the reliance of these may be questioned. It is very significant, however, that the gametic ratios of the coupling and the repulsion values show such a close correspondence, in the former case $n=3,9$, in the latter $n=3,8$. It is very improbable, indeed, that the two different cases should give extreme deviations from a common middle value in the same direction in both cases. The allowed range of fluctuation diminishes greatly in such cases, and the linkage values obtained from the numerical relations of the different phenotypes, therefore, must be considered more reliable than is indicated in the above control.

The gametic ratio may be determined in still another way. Technical difficulties in raising a sufficient number of individuals in the back crosses render this method impossible in the case of *Lupinus angustifolius*. The gametic ratio may be calculated directly from the proportions between the families of certain segregating types in F_3 , and the result of the F_2 -analysis may be checked with the help of these proportion values. The linkage values have been determined in certain cases in this way by MULLER (1916) in *Drosophila*.

	AB	Ab	aB	ab		AB	Ab	aB	ab
	1	n	n	1		n	1	1	n
AB	1	n	n	1	AB	n^2	n	n	n^2
Ab	n	n^2	n^2	n	Ab	n	1	1	n
aB	n	n^2	n^2	n	aB	n	1	1	n
ab	1	n	n	1	ab	n^2	n	n	n^2
	a					b			

Fig. 2.

By the usual checker scheme it is easy to demonstrate that in cases of repulsion (fig. 2 a) the relation between the number of families in the centre and in the middle parts of the periphery is a simple and constant function of the gametic ratio. The repulsion segregates and the constant simplex recessives belong to the centre — their number is $4n^2$ — the families of different types segregating in 3 : 1, $8n$ in number, belong to the middle parts of the periphery. Thus the relation between these two groups is $\frac{4n^2}{8n} = \frac{n}{2}$. The corner-families of the periphery do not need to be considered, partly because of the easy task to recognize them in the F_3 -analysis and to exclude them from the calculations, partly because of the small number of these families which makes possible their neglecting especially in cases of close linkage, that is when n attains large values.

The same results are arrived at in cases of coupling with regard to families occupying other places in the quadrate (fig. 2 b). Here it is the families in the centre which may be omitted. The corner-families give the numerator $4n^2$, while the denominator, $8n$, is still the characteristic value of the middle parts of the periphery. Thus in cases of

coupling the corners and the middle parts of the periphery are compared, and the relation between these groups is $\frac{n}{2}$ as said before. Other combinations, naturally, may be made within the F_3 -families in order to determine the relative gametic ratio, but this is the most suitable partly because of its simplicity, partly because of the fact that only the largest groups are used, which insure the most reliable results. Furthermore, the method makes use of families resulting from coupling as well as from repulsion at the same time. A summary of the

TABLE 3.

List of the number of families of different segregating types in F_3 and F_4 from cross 9.

Type of segreg. in parent fam.	Colour of parent plant	Kinds of phenotypes in the families	Types of repulsion ratios	$4n^2$	$8n$	other groups
Repul- sion	Blue	Blue	Constant	—	—	1
	»	Blue + violet + bl. red + pure red	Coupling	—	—	6
	»	» » » »	Repulsion	43	—	—
	»	Blue + violet	3 : 1	—	18	—
	»	» + bluish red	3 : 1	—	19	—
	Violet	Violet	Constant	28	—	—
	»	Violet + pure red	3 : 1	—	18	—
	Bluish red	Bluish red	Constant	32	—	—
	»	Bluish red + pure red	3 : 1	—	13	—
	Pure red	Pure red	Constant	—	—	2
		Total		103	68	9
Coup- ling	Blue	Blue	Constant	6	—	—
	»	Blue + violet + bl. red + pure red	Coupling	9	—	—
	»	» » » »	Repulsion	—	—	1
	»	Blue + violet	3 : 1	—	4	—
	»	Blue + bluish red	3 : 1	—	2	—
	Violet	Violet	Constant	—	—	1
	»	Violet + pure red	3 : 1	—	1	—
	Bluish red	Bluish red	Constant	—	—	4
	»	Bluish red + pure red	3 : 1	—	1	—
	Pure red	Pure red	Constant	5 ¹	—	—
		Total		20	8	8
		General total		123	77	—

¹ Fictitious value = $\frac{\text{constant} + \text{coupling}}{3} = \frac{6 + 9}{3}$ (see text)

families belonging to different segregating types (F_3 and F_4) of cross 9 is given in table 3. Column 1 tabulates the families, which in the case of repulsion on the part of the parent families occupy the centre of the quadrate, and those which in cases of coupling occupy the corners. Thus all the families forming the denominator are tabulated in this column. Column 2 tabulates the families segregating in the ratio 3 : 1, that is the numerator. For sake of completeness the rest of the families is put together in col. 3, which is not used, however, when the linkage values are calculated. The total of the first col. is 123 families, the second is 76, and the result is the following equation:

$$\frac{n}{2} = \frac{123}{76}; n = 3,2$$

The standard error of the proportions 123 : 76 is $\pm 4,85$. This figure, simple or taken three times, added or subtracted, gives rise to displacements in the proportions of the families and corresponding changes in the gametic ratios as seen in the following:

$$\begin{array}{l} 1M \left\{ \begin{array}{ll} 127,85 : 71,15 & \text{corresponding to } n = 3,6 \\ 118,15 : 80,55 & \text{» » } n = 2,9 \end{array} \right. \\ 3M \left\{ \begin{array}{ll} 137,55 : 61,45 & \text{» » } n = 4,5 \\ 108,45 : 90,55 & \text{» » } n = 2,4 \end{array} \right. \end{array}$$

It should be stated that with regard to the constant pure red families showing coupling a fictitious value of their number has been used for the following reason. As the number of the pure red plants had been determined already in F_2 , and as it further appeared certain that all should show constancy in F_3 , no need of raising F_3 -families from them was present. In order to be able to use the formula, however, a value representing one third of the number of the corner-families left has been used, in this case 5, which stands in about the same relation to the sum of the rest of the families as the pure reds to the sum of the rest of the phenotypes in F_2 of the parent family no. 92—19. This procedure should be followed generally, partly because of the avoidance of an unconscious selection in the taking out of the parent plants, which this procedure makes possible, partly because of the nullification of an eventual poor vitality of the double recessives.

The degree of the repulsion of this cross has been found to be 1 : 3,8 by means of the F_2 -method, and the F_3 -method gives the result

1:3₂. This must be considered a good correspondence in view of the standard error calculations. The value of n lies probably between 3 and 4 corresponding to the crossover percentage 25—20. On account of the numerical relations of the F_3 -generation and the relatively good correspondence between the results of the coupling segregation as well as of the repulsion segregation it must be concluded that the value of n in all probability lies close to 3₅, which value corresponds to the crossover percentage 22₂.

The use of back crosses in the determination of the gametic ratio in *Lupinus angustifolius* is for many reasons technically difficult, as said before. The number of seeds in each pod is very limited, so a very great number of crosses had to be made to obtain sufficient quantities of seed. Numerous pollinations, in addition, do not succeed. At best, seeds from 4—5 flowers only are obtained, from each plant.

It may be of some interest to make a comparison between the precision and the practical advantages of the F_2 - and F_3 -methods, as similar conditions prevail in other genera, which have furnished important data with regard to linkage, as *Lathyrus*, *Triticum*, *Hordeum*. The F_3 -method follows a more direct course, and the results would seem to be more reliable for this reason. This is particularly true in cases of repulsion, where the group of double recessives always is relatively small and consequently oftentimes doubtful. The association coefficient becomes uncertain in a corresponding degree. A fluctuation involving one or a few individuals may influence the ass. coeff. and displace the results considerably. The F_3 -method offers great advantage in this respect as no attention need to be paid to the frequency of the numerically small groups. The advantage of this method is most clearly seen in cases where the number of the individuals in the recessive groups is decreased through poor vitality. MULLER made use of the F_3 -method in *Drosophila* (1916, p. 354) in order to escape these difficulties. The advantage of the F_3 -method is less obvious in the cross 9, just discussed, as the linkage here is relatively loose. It is better seen in cross 8 where close linkage is present, as already stated (pag. 316).

Although the F_3 -method certainly is to be preferred, especially in case of a segregation of repulsion type, great practical difficulties may be encountered in the raising of a sufficient number of families necessitating much work and expense. It may therefore be of some interest to mention a short cut in the use of the F_3 -method, which I intend

to employ in the future in *Lupinus*. It is labour-saving, and an analysis comprehensively enough may be made easier.

The difficulties in the raising of a sufficient number of F_3 -families are remarkably reduced if the number of individuals in each family is intentionally decreased to a minimum. The question is to distinguish between the families segregating in the ratio 3 : 1 on the one hand, and the constant and the di-hybrids of different kinds on the other. A number of 32 individuals at the most in each family is required to make a sure discrimination — provided that the recessives are fully vital. The probability that a heterozygous plant shall give 32 dominants in succession is as 1 : 10000. Thus even if an analysis is made comprising up to 10000 families probably only one family would be found to be erroneously grouped. Such a wrong grouping is of no account, however, as the rarer family-group has occurred often, even in cases of close linkage. There is no need of so great a precision in the case of low or moderately high linkage values. If the number of individuals in the F_3 -families are 24 throughout, one in 10000 families may be considered wrongly grouped. This precision may often be considered sufficiently accurate; indeed, a number of 20 individuals in each family will be sufficient in certain cases. The number of plants in each family may thus be limited to 20—32 according to conditions.

An F_3 -analysis of such a great number of families may mean too great a work to many, even if the task has been made easier through the limitations proposed above. However, the number of the individuals may be still lowered in the following way. The sowing and the investigation of each family take place in different sets; only a few seeds of each family are sown the first time, for instance 4, the second portion may include another set of 4 seeds, and the rest, up to a maximum of 32 for instance, is sown the third time (if not a fourth set is tried). Families at once classed with certain segregating types — different in coupling and in repulsion — already at the first sowing do not need to be repeated in the sowings made later. The last sowing includes then mainly *one* of the two groups of segregating types together with a small number of segregates belonging to the other group, which by a chance failed to expose its place in the first sowings made. The extent of the work depends apparently on the number of families left for the last sowings. Much labour-saving may therefore be made if the parent plants of the F_3 -families are selected in the appropriate way. From the checker scheme, fig. 2 a, it is at once seen that it is inexpedient to use simplex recessive F_2 -

plants as parent plants in the case of repulsion. The majority of these are constants — the greater the repulsion, the greater their number — and the main part of the families from these plants would be left for the last sowing. Thus the simplex recessives should be excluded as parent plants in the case of repulsion; these should instead be taken from the duplex dominant group. The last sowing will then include only the 3:1-segregates, now in minority, together with the very few constant duplex dominants, and the di-hybrids, which have not yet shown their di-hybrid character. In the case of coupling, on the other hand, only the simplex recessive F_2 -plants are selected; the experiment gets freed from the constant double dominants as well as from the families passing into repulsion. The total number of the individuals necessary in F_2 may be limited considerably in this way. Although seemingly paradoxical the statement holds true that the greater the coupling the easier the work, due to the fewness of the groups necessary in the last sowing.

The advantage of this procedure may be readily demonstrated by the following example. We assume that the gametic ratio in a case of repulsion, otherwise identical with cross 9, is 1 : 30 : 30 : 1. We assume, further, that F_2 segregates in 1000 blue, 500 violet and 500 bluish red for instance. The gametic ratio, then, is not to be found from the numerical relations in F_2 ; the F_3 -analysis becomes necessary. As the fixing of the gametic ratio becomes surer the greater the number of families, 480 families for instance are raised. The following total number of plants has to be raised in the case of such an analysis. As it is a case of repulsion only blue individuals are used as parent plants. The constant blue group from the upper left corner of the periphery of the scheme and the groups passing into coupling, which groups in all represent the expectation $\frac{3}{4}$ family in a total of 480 families, are ignored. The families from the middle groups of the periphery, segregating in the ratio 3 : 1, have naturally to be included in the last sowing. Their number should be 30. The total number of individuals to be raised becomes 960, as 32 seeds from each of these 30 parent plants have to be sown successively. A number of 450 di-hybrid families, segregating 1 : 2 : 1, has further to be expected. The first sowing of these will then include 1800 plants in all. According to probability 43 % of these families will show their di-hybrid character, while 57 % have to be sown again. This makes 256 families and 1024 plants. When the result is combined with the numerical relations obtained in the first sowing 65 % of these 256 families are found to

be di-hybrids. Thus only 90 families are left for the third sowing. As 4 plants from each family have to be sown 360 plants are needed. At least 90 % of these 90 families must be assumed to have showed di-hybrid segregation (the figure has not been determined exactly as the results of the previous sowings have to be considered, which complicate the calculations), and only 9 such families have not yet been identified. The remaining 20 seeds from each family are now sown. The maximum number, 32, is reached, increasing the total with 180 plants. The following summarizes the results.

30 families segregating 3 : 1, each with 32 individuals....					960 plants						
1 sowing of 450 di-hybrid families, each with 4 individuals					1800 »						
2	»	»	256	»	»	»	»	4	»	1024	»
3	»	»	90	»	»	»	»	4	»	360	»
4	»	»	9	»	»	»	»	20	»	180	»
										Total	4324 plants

The total number of plants becomes less, viz. 3790, in the case of coupling where the distinction is to be made between the constant and the 3:1-segregating families, and where only simplex recessive plants are taken as parent plants.

If $\frac{1}{2}$ blue, $\frac{1}{4}$ violet and $\frac{1}{4}$ bluish red had been selected according to the numerical relations in F_2 instead of following the procedure just discussed a number of 9000 or 10000 plants had to be raised. If the number had been limited to 32 plants only in each family, and, furthermore, if parent plants had been grown from every phenotypic group without any portioning out of the investigation on different sowings a number of 15360 had to be raised.

In the case of a repulsion of 1 : 30 only one double recessive is expected in about 4000 individuals, which is just about the number required according to the F_3 -method. This shows clearly the advantage of the F_3 -method compared to the F_2 -method. That it also advantageously competes with the back-cross method is seen from the fact that about 1000 plants had to be raised to secure a fixing of the gametic ratio with the same degree of accuracy. The raising of such a great number of hybrids must be considered a heavy task in the case of poor seeders and difficult pollination.

It is clear that the above procedure saves much labour, and that an F_3 -analysis of sufficient extent is made easier. This must be of great value in cases where back crossing is difficult or impossible to perform on a large scale as, for instance, in the families Leguminosae

The result is quite normal with regard to the appearance of expected phenotypes as, contrary to the results obtained in cross 8, a dilution type of the fully coloured parent type (bluish red) originates. The numerical relations, however, do not agree well with the ratio 9 : 3 : 3 : 1. The discordance is still greater in F_3 , which gives the following di-hybrid segregation numbers.

	277 blue	:	121 ting. blue	:	102 bl. red	:	7 ting. red
expected							
acc. to 9:3:3:1	285, ₃		95, ₁		95, ₁		31, ₇
D/M	8, ₃ /11, ₂ =0, ₇₄		25, ₉ /8, ₈ =2, ₉₄		6, ₉ /8, ₈ =0, ₇₈		24, ₇ /5, ₅ =4, ₄₉

The deficit in the tinged red group is remarkably great, and the deviation does not lie within the allowed range. The deviations become more appreciated when the di-hybrid segregations in F_2 and F_3 are summed up:

	354 blue	:	153 ting. blue	:	143 bl. red	:	13 ting. red
expected							
acc. to 9:3:3:1	372, ₉		124, ₃		124, ₃		41, ₄
D/M	18, ₉ /12, ₈ =1, ₄₈		28, ₇ /10, ₀ =2, ₈₇		18, ₇ /10, ₀ =1, ₈₇		28, ₄ /6, ₁ =4, ₆₆

There is a deficit in the blue and the tinged red groups, while there are too many in the tinged blue and in the bluish red groups. It is, therefore, very probable that we here have another case of repulsion, even if an ordinary di-hybrid Mendelian segregation with irregular segregation numbers would explain the matter. The great deficit in the recessives should then be traced back to poor vitality, although nothing else favours this assumption. Unfortunately, the analysis has not been carried out on a scale sufficient to guarantee a definite decision. The relatively small number of F_3 -families due to diseases, which happened to damage the F_2 -plots of this cross more than other plots, is particularly to be deplored. Otherwise the question could have been easily settled especially through the occurrence of families showing segregation characteristic of »coupling». It is true, one family has probably to be referred to this type, viz. no. 287—19, but the small number of individuals precludes a decision. The results of the segregation are by no means conclusive but some of the facts agree well with the assumption of the existence of repulsion in this cross, and this is further strengthened by the fact that each of the factors in question are coupled with a third to be discussed in the following.

The degree of the linkage is seen from table 4. A scrutiny of the reliability of these figures — in view of the relation between the

TABLE 4.

Reference number	Generation	Frequency of phenotypes					Ass. coeff. of observed phenotypic ratio	Standard error M	Ass. coeff. of calcul. phenotypic ratio	Gametic ratio	Percentage of crossing over
		CD	Cd	cD	cd	Total					
Repulsion.											
1	F_2	77	32	41	6	156	0,4792	0,1878	0,4752	1:1,8	35,7
2	F_3	249	119	96	5	462	0,8034	0,0837	0,8074	1:3,6	21,7
3	$F_2 + F_3$	326	151	137	11	625	0,7046	0,0827	0,7003	1:2,7	27,0
Coupling.											
4	F_3	28	2	6	2	38	0,6470	0,3188	0,6407	2,3:1	33,3

zygotic groups to be expected in case of di-hybrid numbers in general, according to the facts stated on pag. 318 — at once shows that the numerical relations of nos. 1, 2 and 4 are very doubtful. The inner groups are rather unequal in all three cases, and the proportions between the outer terms are also irregular. The relations between $CD - (Cd + cD)$ and cd are listed in the following and compared to the relations to be expected at the numbers secured by the summing up of these two quantities.

1. $4:6 = 10$ expected $7,5:2,5$
2. $34:5 = 39$ » $29,25:9,75$
4. $20:2 = 22$ » $16,5:5,5$

The irregularities of the repulsion values in F_2 and F_3 become smoothed out when summed up, and the numerical relations (ref. no. 3) representing the sum of the repulsion values of both generations agree rather satisfactory with the general values just listed. The gametic ratio obtained from the summed up values is 1:2,7. The standard error of the association coefficient of the observed values in ref. no. 3 is 0,0826, which indicates a fluctuation of the gametic ratio between 1:2,3 and 1:3,4, or — if the threefold standard error is used — between 1:1,8 and 1:8. The uncertainty is thus seen to be very great. The deviations obtained between the different values of the gametic ratio lie within the allowed range.

The determination of the gametic ratio according to the F_3 -method gives the following result. The number of families of different segregating types are listed in table 5; they are grouped in the same way

Cross 9 shows that the meeting of the factors *B* and *V* is necessary if deep blue colour shall result. Bluish red has been crossed here with violet, and F_1 is blue. The one type alone can not have carried the determiners of the blue colour as this colour is dominant over bluish red as well as over violet; these must have been distributed in the both parent types. The assumption of quite other factors than those for bluish red and violet bringing about the synthesis would also explain the matter, and the mere occurrence of a blue coloured F_1 -generation in this cross is no adequate proof. The segregation in F_2 and F_3 would then have been another, however. Bluish red and violet individuals are found in the same number in F_2 , and violets never originate from bluish reds in F_3 , or vice versa. Plants of these colours never carry more than one of the determiners in question; the flower is always blue in the plants carrying both determiners. Thus this colour does not depend on a specific factor; it is on the contrary a compound character genetically, and the factors involved are the ones denoted for bluish red and violet.

The effect of these factors, on the other hand, depends on the factor for pure red or *R*. The *B*-factor induces a faint blue coloration in the red flower, as well as in the brownish red vegetative parts of the pure red type. It is apparently a bluing factor. It is another example of a factor with bluing effect in red flowers so commonly found in widely different genera. An examination of the bluish red flowers seems to show, however, that the red pigment has been transformed into blue pigment only in a limited number of cells. The red ground colour remains unchanged to a great extent, and only certain parts are more or less covered with a blue tinge. The bluish red colour, then, is not a specific colour but a combination of blue and red. It seems questionable only for this reason if it is to be compared with the purple colour in *Lathyrus*, for instance, a parallel which lies close at hand. The blue coloration spreads more diffusely in the flower with age, and the similarity with purple colour becomes greater. That the *B*-factor is a bluing factor is clearly seen in its relation to the seed colour. It has been assumed that the purpose of the *B*-factor with regard to seed colour is to transfer the rust-brown pigment of the pure red type into earth-brown. The examination of immature seeds shows, however, that the effect in fact also here is a blue coloration. Seeds still enclosed in the yellow but not yet dry pods show a red-violet coloration of the coloured parts in the rust-brown type, while the same parts in the earth-brown type are

blue. While the bluing effect of the *B*-factor is rather weak in the flowers; requiring the co-operation of the *C*-factor in order to produce the deep blue colour, no such co-operation is required in the case of the seeds. Blue seed colour is found in bluish red plants as well as in quite blue plants, and the colour has the same intensity in both cases; it is not possible to tell the particular type of flower colour from the colour of the seeds.

The *C*-factor transfers the pure red flowers colour into violet. It is not a question of a mixing in this case, apparently; the ground colour is probably wholly transferred into a new pigment. It is true that a faint tinge of blue is sometimes found also here, but the ground is always violet and not red. The *V*-factor does not influence the seed colour, and the rust-brown colour remains therefore quite unchanged.

It is of great interest to draw a parallel between the flower colours of different species and genera even if the accuracy of such a parallel becomes doubtful for want of chemical investigations. The studies of WILLSTÄTTER in the chemistry of the flower colours have showed a remarkable correspondence in the composition of the pigments in different genera. It appears very probable that the flower colour of *Lupinus*, although not analyzed chemically, presents similarities with numerous genera investigated by WILLSTÄTTER as to the composition of the pigments. The colour scale of *Lupinus* corresponds exactly with the scale found in *Centaurea* and *Delphinium*; the assumption of a correspondence as well in the chemistry of the pigments between *Lupinus* and the latter genera does not seem to be too bold.

The following points of comparison between the genetical scheme found in *Lupinus* and the chemical scheme of WILLSTÄTTER would then be held forth. The *R*-factor in the pure red type would be made responsible for the occurrence of acid cell sap and for the presence of the anthocyanin in the form of a homologue to the oxonium salt, which has been found to form the red pigment in *Centaurea*. The *V*-factor would cause a basic influence on the cell sap neutralizing the acid of the pure red type and liberating the anthocyanin, which now gives violet colour. The *B*-factor would also cause basic effect, as the blue colour found with this factor and caused by a calcium salt with the anthocyanin as a base requires alkaline cell sap. The *B*-factor alone, however, produces alkaline cell sap only in some of the cells of the flower. The result is a blue tinge on red ground, which gives rise to the compound colour bluish red. It seems quite natural

that the deep blue colour is the result of the co-operation of the two factors *B* and *V*, as both factors seem to cause alkalinity and as, further, the effect of the *V*-factor apparently pervades the whole flower. It is, indeed, of great interest that the neutral colour violet genetically is an intermediate between red and blue, between acid and alkaline colour. However, the schematic outline of the hypothesis of WILLSTÄTTER has its limitations; it was found that the red pigment of the rose-coloured form of *Centaurea* was not a cyanin variety, although expected, but a related still different pigment called pelargonin. This shows that the generalization made in the above must be looked upon with reservation.

A genetical analysis of a plant with similar colour scale has not yet been made, so far as I know. Deep blue colour together with violet and red do not occur, as a rule, in the plants more fully investigated in this respect (*Antirrhinum*, *Matthiola*, *Mirabilis*, *Papaver* etc.). In other cases a variety is found to be blue, being in these cases recessive, and not the type (*Lathyrus*, *Phlox*, *Anagallis*). The phenotypic difference between *Lupinus* and the latter genera would answer to the lack on the part of these of one link in the factorial chain of *Lupinus*. Purple in *Lathyrus* would correspond either to bluish red or violet in *Lupinus*, and *Lathyrus* would lack one or the other of the *Lupinus* factors *B* and *V*, which explains the absence of the blue compound form *BV*.

The necessity of assuming a fundamental factor for the appearance of colour on the whole is seen from crosses 6 and 7. White has been crossed in both these crosses with bluish red and violet respectively. F_1 is blue in both cases. With the knowledge of the constitution of the blue colour in mind the white parent plant must be assumed to have had at least one of the complementary factors for blue colour. We have, then, to assume that the white colour was due to the absence of a fundamental factor necessary for the development of bluish red as well as of violet, and thus also of the blue colour. Cross 5, white $\times$ tinged blue, favours also the idea of such a fundamental factor. However, it is not necessary to explain the blue colour in F_1 in this way. The tinged blue parent has indeed all determiners for blue colour and lacks only the full colour factor, which is introduced, however, by the white parent. It would be possible to assume that the white parent was devoid of all colour factors, and that it showed white colour because of this circumstance and not because of the absence of a fundamental factor only. The segregation in F_2 , however, would then have

turned out differently and brought forth violet as well as bluish red plants. This is not the case. Only blue, tinged blue and white are present, and this shows that the white parent had all determiners for blue colour, while the necessary fundamental factor was absent. It has been assumed that the colour factor for pure red or *R*, which remains when the factors for bluish red and violet have been eliminated, should represent the fundamental factor just discussed. This is in accordance with all the results of the segregations, as pure red is the first link in the colour chain and as no indication of complementary fundamental factors has been obtained so far. The genus *Lupinus* would then form an exception similar to *Pisum*, as the presence of *two* fundamental factors has been found to be the rule in most genera carefully investigated. The most probable thing is, however, that two fundamental factors are present in these as well as in other genera, although only one has been established because of the use of whites lines, where only one of the factors, and always the same, has been present.

That this is so in *Lupinus* becomes very probable when it is remembered that only few lines of the genus have been investigated; my crosses comprise only two white flowered lines. That the same state of things has been found in the classic genus *Pisum* is a fact of greater significance. The diverging genetical constitution may indicate a deviating course of the formation of the colour. It would therefore be of great interest to follow up this side of the matter through an extensive study of white flowered lines of both genera.

The white colour in *Lupinus* shows another peculiarity, which distinguishes it from several other plants. The white forms of *Lupinus ang.* do not represent pure albino forms as is generally the case, in *Lathyrus* and *Antirrhinum* for instance. Certainly, they lack every trace of colour in all vegetative parts just as these, and buds and young flowers are pure white. Indeed, even older flowers may lack colour in certain cases. The flowers become coloured with age as a rule, however, and this coloration is sometimes very marked. Furthermore, the seeds of the white type are generally faint brown marbled with a rust-brown scar at the hilum. The same coloration of the seeds is found in *Pisum*, which also has »ghoast» coloured white seeds. Observations made the last year show that the shade of the colour is not the same in all whites, but varies with the colour factors present in a »latent» state. The outer conditions have apparently greatly favoured the development of the colour this year (unsui-

table weather conditions during the early vegetative season kept the young plants small and poorly developed for a long time). The distinct development of the colour made it easy to notice the difference in F_3 of cross 7 between the whites in the families segregating in blue and white, and the whites in the families segregating bluish red and white. The former were more bluish than the latter. A classification of the different white types coexisting in families of di-hybrid segregation was then tried. 62 white individuals from such families were divided in two groups. In the first group individuals were put having the same colour as the one noted in the families segregating in blue and white; the second group includes whites coloured in the same shade as in families segregating in bluish red and white. The numbers of individuals in these groups were 41 : 21; the expected proportions, according to the ratio 3 : 1, is 46.5 : 15.5. Thus it is clear that the factors B and V also determine the colour of the white types.

The question now becomes of interest whether the phenotypical differences between the white types in *Lupinus* and other genera indicate a difference as well in the factorial constitution. It is possible that the modifying factors B and V have a colour effect of their own without the co-operation of the fundamental factor. It is also possible that the white type does not represent an albino form but a dilution form, brought into existence by the absence of a full colour factor. These hypotheses are probably both erroneous. The colouration in question certainly does not indicate a diverging factorial constitution; it depends rather on purely physiological peculiarities. The coloration of the white flower belongs probably to the same category of phenomena as the shading of the colour noticeable in the coloured types in *Lupinus* when old. In the genus *Anchusa*, where also the colour of the flowers changes with age, no coloration of the white type is seen. Thus there are marked differences between the white types of *Lupinus* and of *Anchusa*; it does not invalidate, however, the parallel drawn in the above with regard to the shading of the coloured and white types. It is probably due to physiological changes in the tissues of the plant. A change in the permeability of the vacuolar membrane suggests itself. It is conceivable that a substance entering the vacuole may be endowed with the power to change the anthocyan of the cell sap, thus compensating the colour factor, which has been found to be absent. If we, accepting WHELDALÉ's hypothesis of colour formation, assume the presence in the white type of a factor for chromogen and the absence of the factor for the development of

oxydase in the cell sap no difficulty is met with in explaining the old age coloration through the entrance into the vacuole of a protoplasmatic oxydase, which in co-operation with the vacuolar chromogen develops the colour. (Cp. the hypothesis formulated by JONES with regard to the coloration of white types, JONES 1913, ref. in SCHIEMANN 1915). The following facts, however, should be noted. The shade of the old age coloration depends on the nature of the modifying factors present (*B* and *V*), as said before. WHELDALE regards the effect of the modifying factors as a continued oxidation of the products of the primary oxidation through specific enzymes. Such enzymes must therefore in this case have been present in the cell sap of a white plant with the constitution *rBV*, for instance. Thus these enzymes would not be able to compensate the enzyme of the fundamental *R*-factor; this should be attained, however, by a foreign enzyme (the *R*-factor is absent), which enters the vacuole — a rather strange phenomenon.

This hypothesis of the changes in the permeability of the vacuolar membrane agrees also with the interpretation of the nature of the white types given by SCHIEMANN. This is based on the facts established by WILLSTÄTTER that the anthocyan colours have a tendency to form colourless isomeres, and that certain substances have a power to check this isomerisation. The cell sap would then be assumed to contain anthocyan but of its colourless isomeric variety. A regeneration of the coloured isomere would be made possible through the successive changes in the chemical composition of the cell sap, provided that the above discussed changes in the permeability of the vacuolar membrane take place.

The above hypothesis is mentioned only as an example. Several other circumstances with similar effect may be conceived of as factors in the colour development of the flower. Furthermore, the change in the composition of the cell sap may just as well be accomplished through the withdrawal of a substance. It appears probable, however, that the development of colour in the white types follows this course or a similar one.

In the scheme proposed the *F*-factor has been assumed to have a general effect. It has also been assumed that the occurrence of diluted forms of the colours, determined by the presence of the other factors, is due to the absence of this *F*-factor. Dilution of blue has been found in all crosses, where dilution forms have been found at all, and full coloured blue has always been found at the same time

(crosses 4, 5, 8 and 10). That the F -factor also influences the bluish red colour ($RRBB$) is seen from cross 10, where tinged red originates as a product of recombination. It is true, no tinged form of violet has yet been found, but this is probably due to close linkage. It is, therefore, a difficult task to succeed in obtaining this form from the material at hand, although probably not unrealizable. The relation of the F -factor to the pure red factor is not yet known.

I have succeeded in determining the Ff -plants in the case of blue colour, as all F_1 -generations involving the F -factor have showed a weak dilution of the blue colour. This dilution is so weak, however, that it is observed only in an F_1 -plot, where all plants have the same diluted shading contrasting with the next, fully coloured plot. However, great difficulties are experienced in separating the different types in

TABLE 6.

Colour of parent plant		FF		Ff	
		Constant blue	Mono-hybrid, without dil.	Di-hybrid segregation	Mono-hybrid, without dil.
Blue.....	cross 5	7	6	4	1
	cross 10	2	4	5	0
	total	9	10	9	1
Slightly diluted blue	cross 5	0	0	6	2
	cross 10	0	0	6	1
	total	0	0	12	3

F_2 , where homo- and heterozygotically blue individuals are mixed. They have been listed with the pure blue type for this reason, when examined. I have classified the individuals as accurately as possible in blue and slightly diluted blue in the taking out of the parent plants for the F_3 -families in crosses 5 and 10. The relation between the phenotype and the genotype in this case is shown in table 6. The result is significant. Individuals with the full colour factor in duplex stage have in no case been listed as slightly diluted blue but always as blue, but the Ff -individuals have been listed in 10 cases as blue and in 15 cases as slightly diluted blue. Presuming complete dominance of the F -factor these facts would be explained by the assumption that the FF -plants do not become modified towards diluted blue, while the Ff -individuals do become modified in this way. If the F -factor predominates only, the facts become explained by the

assumption that the colour of the *Ff*-plants may be intensified towards full blue colour. That is to say that the *Ff*-stage is more modifiable and therefore varies more than the *FF*-stage. Thus the transition to the *Ff*-stage is noticeable, though the main part of the colour dilution is due to the complete absence of the *F*-factor. This is true only of the blue colour, however, but not of the bluish-red type. I have not yet been able to distinguish the *vvFf*-types from the *vvFF*-types in spite of careful observations. The case of incomplete dominance mentioned is the only one found in *Lupinus*; the dominance is complete as to all other characters to all seeming.

The pleiotropic effect of the colour factors have already been mentioned; they give rise to coloration not only in the flowers but also in the sepals, in the cotyledons, in the axis etc. The *R*- and *B*-factors also influence the colour of the seed. The phenomenon is very common. WHELDALE cites 14 different genera, where a genetical connection between flower colour and anthocyan in vegetative parts has been found; they do not all represent pleiotropic phenomena, however. Among these genera intensifying factors and full colour factors have been found in *Antirrhinum* and *Primula*, and these factors influence also the coloration of the vegetative parts. I have not been able to find any data as to the eventual weakening of the colour in the leaf axils in *Lathyrus*, when the full colour factor is absent; this is the case in *Pisum*, however. A weakening of the colour of the flower is probably in most cases followed by a weakening of the colour of the vegetative parts. This is not the case in *Lupinus*, however. While the absence of the *F*-factor gives rise to a very marked dilution of the flower colour, which almost disappears in the beak of the keel, no trace of such a dilution is found in the vegetative parts of the plant. The faint coloured tinged red type has just as dark earth-brown seeds as the deep blue coloured type, and the colour of the bracts is identical with the colour of the bracts in the full coloured bluish red type. Thus a factor regulating the colour intensity of another pleiotropic factor influences the effect of this factor to different degrees in different organs. In one organ, e. g. the beak of the keel, every trace of the colour disappears although normally coloured intensively; in another organ no change in the colour is seen in spite of the fact that the colour often is very weak in the full coloured type, for instance in the stem, where only a faint tinge of colour covers the green ground. In still other organs, e. g. standard and wings, the

colour is weakened. The full colour factor, then, is not pleiotropic to the same degree as the colour factors.

Another interesting point of some weight has been mentioned with regard to the pleiotropism of the colour factors, which illustrates the common phenomenon that the effect of a pleiotropic factor differs in different organs. Two different cases of pleiotropism may be found. In the first case the factor has the same effect in different organs; blue colour in the flower as well as in vegetative parts as in *Lupinus*, and zygomorphism in both calyx and corolla as in *Antirrhinum*, according to BAUR, are good examples of this first kind. In the second case the effect of the factor varies in different degrees in different organs. Many examples are known of this kind, as for instance blue flower colour and earth-brown seed colour (*Lupinus*), and the flower colour and the width of the petals (TAMMES in *Linum*) to mention a more surprising case. The terms *isophene* and *heterophene pleiotropism* are proposed for these two kinds of pleiotropism. The isophene pleiotropism is clear enough but the heterophene kind present sometimes very unexpected combinations. However, the above mentioned case of heterophene pleiotropism in *Lupinus* (blue flower colour and earth-brown seed colour) is as readily explained as the isophene pleiotropism. When the immature seed is examined it is seen, as said before, that the earth-brown coloration induced by the factor *B*, really is a blue coloration, which passes into the earth-brown colour in consequence of maturing processes. In this case a morphological observation suffices to show that the means of action of the pleiotropic factor are the same in the both organs; the cause of the different phenotypical effects of the factor is wholly due to different conditions prevailing in the both organs. Another illustrative example of this kind of pleiotropism is the interpretation given by HERIBERT-NILSSON to account for the cracked bark and the curved leaves of certain segregates of *Salix*-bastards; both these characters are traced back to an abnormal rate of growth of the vascular cylinder (the xylem). A careful anatomical, physiological or chemical analysis of marked heterophene pleiotropism shall probably in many cases similarly bring out the fact that the differences are to be traced back to a common cause. The phenotypical diversity, of course, is due to one and the same undifferentiated genetical factor in these cases. As long as the real nature of the factors is unknown, there is no use of discussing the question, whether the genetical factor should be assumed to be undifferentiated and simple in the case of characters showing gene-

tical affinity but not yet proved to be related physiologically or chemically. It is, naturally, of great importance to know if the morphological effect of the one and same factor is due to circumstances conditioned by different developmental stages of the plant, different conditions prevailing in the different organs of the plant, or, on the contrary, if the factor itself is differentiated in some way. Thus the study of pleiotropic phenomena becomes of great weight when the nature of the genetical factors is brought under consideration.

THE SEED COLOUR.

The observations made with regard to the seed colour have been stated in the above in connection with the flower colours; they have been assumed to arise from the same factors due to the pleiotropic nature of these factors. The results of the segregations agree also throughout with this interpretation; they allow also the assumption, however, that specific seed colour factors are closely coupled with the flower colour factors. This is, however, very uncertain, even if certain facts would seem to point in this direction, as for instance the fact the *V*-factor, as well as the full colour factor, do not influence the seed colour. The evidence obtained, however, does not support the giving up of the assumption made, that the flower colour factors also determine the colour of the seeds.

There is one character of the seed colour, however, that has not yet been treated, viz. the marbling. Two types differing in the distribution of the pigment have been described on pag. 303. The earth-brown and the rust-brown colour are either marbled or evenly distributed to full intensity over the whole surface of the seed, with the exception for small distinct white flecks. The marbling is brought about by the scattered distribution of the pigment grains in flecks of irregular form and extent. The colour of these flecks is gray, and small white flecks also occur here. Marbling dominates over non-marbling, and F_2 segregates in the ratio 3 : 1. In cross 2, the only cross where the non-marbled variety has been used, the following results have been obtained:

$$\begin{array}{lcl} 215 \text{ marbled} : 63 \text{ non-marbled} \\ \text{expected} : 208,5 & 69,5 & D/M = \frac{6,5}{7,22} = 0,9. \end{array}$$

The weakening of the colour, which is characteristic of the gray flecks, does not involve an intensifying of the colour in the marbled

areas, which would have been expected had the quantity of the pigment been the same and only the distribution of it unequal. The non-marbled type has exactly the same intensity of colour as the marbled areas of the other type. Thus it is clear that the marbled form is a type weaker in colour than the non-marbled variety. This is of a certain interest, as the marbled type dominates over the non-marbled, according to the results obtained. Thus the *M*-factor, assumed to be responsible for the marbling, exhibits the character of an inhibiting factor. Its effect, however, is limited to the seed; it has no weakening effect on the flower. The marbling factor operates probably quite independent of the flower colour factors. Earth-brown and rust-brown segregate in cross 2 in addition to the marbling character. The di-hybrid segregation is quite normal, and the following values have been obtained:

162 earth-brown, marbled : 47 earth-brown, non-marbled : 53 rust-brown, marbled : 16 rust-brown, non-marbled.

$$\begin{array}{cccc} \text{expected } 156,4 & 52,1 & 52,1 & 17,4 \\ D/M = \frac{5,6}{8,3} = 0,67 & \frac{5,1}{6,5} = 0,78 & \frac{0,9}{6,5} = 0,14 & \frac{1,4}{4,0} = 0,35. \end{array}$$

DISCUSSION OF THE LINKAGE CASES.

The results of the analysis have proved that linkage occurs in two crosses (crosses 8 and 9); the third case of linkage is not absolutely proven (cross 10, bluish red $\times$ tinged blue). The factors involved are *B*, *V* and *F*. The factors coupled in cross 8 are *B* and *F*, in cross 9, *B* and *V*. In cross 10 the factors *V* and *F* are segregating; linkage is thus to be expected also in this case, and the results of the segregation go to show that this view is correct. They do not offer any proof when considered separately, but the fact that each of the factors involved is coupled with a third is conclusive. This being so, theory and the results obtained claim coupling between *V* and *F* as well; the results of the segregation agree well with this assumption, and coupling must be considered proven between the factors *V* and *F*. The factors *B*, *V* and *F* are then situated in the same chromosome, according to the chromosome theory, and their location is assumed to be the following.

According to cross 8 there is very close linkage between the factors *B* and *F*, as in a large material no crossovers (tinged violet) have been obtained. Furthermore, it is very improbable that a continued

analysis of this cross shall establish a crossover percentage higher than 2 %. It will probably be found that the percentage does not exceed 1 %; it will rather fall below this figure, as the chances of a crossover percentage more or less than $\frac{1}{3}$ % are equal, as shown on pag. 316. The factors *B* and *F* are then situated in the closest proximity to each other. According to cross 9 there is linkage between *B* and *V* to a degree corresponding to a crossover percentage of about 22 %. The F_2 -analysis gives the value 20.4, and the F_3 -analysis 23.8. The same crossover percentage is to be expected between the factors *V* and *F* on account of the closeness between the factors *B* and *F*. The values obtained in the case of *V*—*F* are 27 % according to the F_2 -analysis, but 22.2 % according to the F_3 -analysis. The F_3 -result shows thus a striking correspondence with the expectation.

The location of the factors *B*, *V* and *F* in the chromosome may then be demonstrated in the following way.



It should be stated that the mutual location of the proximate factors *B* and *F* is rather gratuitous. All crosses have shown that the fourth flower colour factor, the pure red factor *R*, operates independent of the other factors; it is probably situated in another chromosome. The same is probably true of the marbling factor, which has been shown to be independent of the *B*-factor. The relation of the *M*-factor to the fundamental factor *R* is not yet known.

The coupling between the factors *B* and *V* is particularly interesting. The linkage between these factors is very close; it is perhaps complete, as no crossovers have been found. This might favour the supposition that the present case should be interpreted as a case of multiple allelomorphs. This is erroneous, however, as seen below. The factor denotation used by MORGAN has been adopted in the following presentation of the facts. In order to avoid confusion with the presence-absence system previously employed, only large letters have been used; commas and minus symbols have been used as indices of mutations (see MORGAN 1915 pag. 219).

BV = blue, wildtype; BV' = violet; $B'V$ = bluish red; $B'V'$ = pure red; BV^- = tinged blue; $B'V^-$ = tinged red.

It should be observed that violet colour, for instance, is here called V^- contrary to the presence-absence indices, where violet is denoted, *V*, while *v* denotes bluish red.

According to the above symbols the non-appearance of the tinged violet type in F_2 of cross 8 (tinged blue $\times$ violet) should depend on the fact that the factor producing the dilution of the blue colour and the factor responsible for the violet colour, denoted V^- and V' respectively, are different allelomorphs of the same wildtype factor V . Already the F_1 -result of this cross invalidates this assumption. If correct, no synthesis of the wildtype would be obtained from the cross $BV' \times BV^-$; this synthesis was secured, however.

Another supposition is: that the wildtype factor B , which produces bluish red flower colour when mutating into B' , also should give rise to B^- and B'^- mutations causing the formation of tinged blue (B^-V) and tinged red (B'^-V), is likewise out of question. The non-appearance of tinged violet should then depend on the fact that the V -factor did not mutate in an analogous way. The synthesis of blue in the cross bluish red $\times$ tinged blue ($B'V \times B^-V$) would not be obtained in the case of such a factor construction.

The present case of coupling can not be interpreted as a case of multiple allelomorphs, as seen; it is linkage, and it is perhaps complete.

It is very difficult to determine, however, whether the linkage is partial or complete. The non-appearance of the crossovers may be explained by assuming a corresponding displacement of the linkage value, and the comprehensiveness of the analysis cannot alone decide the matter. Indeed, it is doubtful whether there is such a condition as complete linkage. The term becomes superfluous if only the closest partial linkage conceivable is considered. The question becomes another if the assumption is made that two factors are able to lie side by side in the same chromomere without excluding each other as multiple allelomorphs. The term complete linkage would then imply a definite meaning distinguished from partial linkage, and essentially different from multiple allelomorphism. There is nothing in point of principle to say against such an assumption, as the idea of the chromomeres only expresses the belief of the breaking up of the chromosomes into their constituent elements. The chromomeres represent the smallest parts of division of the chromosomes, which does not imply, however, that they each carry only one factor.

Some of the essential facts of the inheritance of the flower colour and of the seed colour in *Lupinus angustifolius* have now been presented. The definite fixing of the linkage values, the precise relation of the marbling factor to the other factors, and the complete analysis

of the poly-hybrid segregations are facts of interest still uninvestigated. In the crosses analyzed, comprising about 800 families and 27000 individuals, segregation with regard to earliness was also found. The most surprising uniformity prevailed in flowers as well as in vegetative parts for the rest. It is true, the colour of the seed varies remarkably with regard to the intensity and the distribution. The variations are, no doubt, only modifications, as the colour of the seed is very sensitive to environmental conditions. New mutations have not been observed.

On this occasion I wish to express my sincerest thanks to Mr. H. WEIBULL, director of the plant breeding institute at Weibullsholm, Landskrona, for his great kindness of promoting these studies in different ways.

SUMMARY.

1) Seven different types of flower colours and five types of seed colours have been genetically analyzed.

2) One fundamental colour factor has been demonstrated (pure red). A synthesis of blue colour has been obtained from crosses between bluish red and violet flower colours. One »dilution» factor has been found to be present.

3) Pleiotropic correlation has been demonstrated between certain flower and seed colours.

4) Three flower colour factors have been found to form a linkage group. The linkage between two of the factors is very close, if not complete. The other linkage value represent a crossover percentage of about 22 %.

5) The importance of the » F_3 -analysis» in the determination of the linkage values in cases where back crossing is technically difficult has been demonstrated.

6) A method, that greatly facilitates the carrying out of the F_3 -analysis, has been proposed.

7) The colour scale blue-violet-red (blue as the wildtype), now investigated genetically, is phenotypically analogous with the colour scale in the genus *Centaurea* a. o. chemically analyzed by WILLSTÄTTER.

TABLE 7. F_2 of the cross white $\times$ blue n:r 1.

Colour of parent plant	Field-number	Blue	White	Total
Blue	592—18	81	23	104
»	593—18	35	8	43
»	594—18	47	18	65
»	595—18	85	16	101
»	596—18	41	8	49
Total		289	73	362

TABLE 8. F_2 of the cross violet $\times$ blue n:r 2.

Colour of parent plant	Field-number	Blue	Violet	Total
Blue	3339—17	57	19	76
»	3340—17	44	19	63
»	3341—17	9	3	12
»	3342—17	47	14	61
»	3343—17	58	18	76
Total		215	73	288

TABLE 9. F_3 of the cross violet $\times$ blue n:r 2.

Number of parent family	Colour of parent plant	Field-number	Blue	Violet	Total
3339—17.....	Blue	578—18	22	—	22
3340—17.....	Blue	581—18	35	14	49
	»	582—18	60	23	83
	»	583—18	14	2	16
	»	584—18	111	39	150
3342—17.....	»	585—18	60	27	87
	»	586—18	73	17	90
	»	588—18	41	16	57
	»	589—18	62	16	78
Total			456	154	610
3340—17.....	Violet	579—18	—	129	129
	»	580—18	—	43	43
	»	587—18	—	75	75
Total			—	247	247

TABLE 10. F_2 of the cross bluish red $\times$ blue n:r 3.

Colour of parent plant	Field-number	Blue	Bluish red	Total
Blue	610—18	28	5	33
»	611—18	61	25	86
»	612—18	22	5	27
»	613—18	11	5	16
Total		122	40	162

TABLE 11. F_3 of the cross bluish red $\times$ blue n:r 3.

Number of parent family	Colour of parent plant	Field-number	Blue	Bluish red	Total
610—18	Blue	255—19	35	—	35
	»	257—19	33	—	33
	»	267—19	75	—	75
611—18	»	268—19	64	—	64
	»	269—19	23	—	23
	»	276—19	37	—	37
Total			267	—	267
610—18	Blue	253—19	57	17	74
	»	254—19	44	18	62
	»	258—19	23	11	34
	»	260—19	20	5	25
	»	261—19	38	8	46
	»	262—19	23	11	34
	»	263—19	36	13	49
611—18	»	271—19	55	18	73
Total			296	101	397
610—18	Bluish red	264—19	—	20	20
	»	266—19	—	52	52
	»	272—19	—	57	57
611—18	»	273—19	—	34	34
	»	274—19	—	11	11
613—18	»	278—19	—	28	28
Total			—	202	202

TABLE 12. F_2 of the cross *tinged blue* $\times$ *blue* n:r 4.

Colour of parent plant	Field-number	Blue	Tinged blue	Total
Slightly diluted blue	630—18	69	24	93
	631—18	57	21	78
	632—18	82	33	115
	633—18	117	34	151
	634—18	71	26	97
Total		396	138	534

TABLE 13. F_3 of the cross *tinged blue* $\times$ *blue* n:r 4.

Number of parent family	Colour of parent plant	Field-number	Blue	Tinged blue	Total
630—18	Blue ¹	515—19	47	—	47
633—18	»	523—19	54	—	54
		527—19	33	—	33
634—18	»	533—19	52	—	52
		534—19	55	—	55
		538—19	41	—	41
		Total	282	—	282
630—18	Blue ¹	514—19	19	7	26
	»	516—19	36	9	45
	»	517—19	19	5	24
	»	518—19	21	11	32
631—18	»	519—19	27	8	35
	»	524—19	34	8	42
633—18	»	525—19	37	15	52
	»	528—19	35	12	47
	»	529—19	35	9	44
634—18	»	535—19	19	7	26
	»	539—19	42	16	58
		Total	324	107	431
632—18	Tinged blue	521—19	—	8	8
	»	522—19	—	17	17

¹ Because of the difficulty in separating the blue ones and the slightly dil. blue no record is made of the latter in this cross.

Number of parent family	Colour of parent plant	Field-number	Blue	Tinged blue	Total
633—18	Tinged blue	530—19	—	55	55
	»	531—19	—	4	4
	»	541—19	—	15	15
634—18	»	542—19	—	8	8
	»	543—19	—	35	35
	»	544—19	—	5	5
Total			—	147	147

TABLE 14. F_2 of the cross white $\times$ tinged blue n:r 5.

Colour of parent plant	Field-number	Blue	Tinged blue	White	Total
Slightly diluted blue	3332—17	17	6	7	30
	3334—17	22	2	4	28
	3335—17	6	2	0	8
	3336—17	8	0	7	15
Total		53	10	18	81

TABLE 15. F_3 of the cross white $\times$ tinged blue n:r 5.

Number of parent family	Colour of parent plant	Field-number	Blue	Tinged blue	White	Total
3332—17.....	Blue	512—18	20	—	—	20
	»	514—18	23	—	—	23
	»	530—18	54	—	—	54
3334—17.....	»	541—18	31	—	—	31
	»	544—18	42	—	—	42
3335—17.....	»	545—18	37	—	—	37
	»	551—18	54	—	—	54
Total			261	—	—	261
3332—17.....	Blue	516—18	17	3	4	24
	»	517—18	15	5	5	25
	»	519—18	15	5	6	26
	? ¹	527—18	17	10	4	31
	?	528—18	31	13	15	59
3334—17.....	Sl. dil. blue	529—18	11	8	5	24
	»	531—18	26	11	8	45
	»	532—18	29	10	14	53
	»	536—18	13	6	4	23
	Blue	539—18	50	10	23	83
Sl. dil. blue		543—18	18	9	10	37

¹ Not recorded.

Number of parent family	Colour of parent plant	Field-number	Blue	Tinged blue	White	Total
3335—17.....	?	547—18	45	14	13	72
3336—17.....	Sl. dil. blue	552—18	50	23	27	100
		Total	337	127	138	602
3332—17.....	?	509—18	23	3	—	26
	Sl. dil. blue	511—18	38	12	—	50
	?	526—18	15	10	—	25
3334—17.....	Sl. dil. blue	533—18	43	9	—	52
	Blue	540—18	20	6	—	26
3336—17.....	?	548—18	19	8	—	27
		Total	158	48	—	206
3332—17.....	Blue	518—18	18	—	4	22
	»	534—18	61	—	20	81
3334—17.....	»	538—18	39	—	13	52
	»	542—18	33	—	11	44
3336—17.....	»	550—18	38	—	14	52
	»	553—18	35	—	16	51
		Total	224	—	78	302
3332—17.....	Tinged blue	503—18	—	34	—	34
	»	508—18	—	54	—	54
3334—17.....	»	523—18	—	27	—	27
3335—17.....	?	546—18	—	68	—	68
3336—17.....	?	549—18	—	33	—	33
		Total	—	216	—	216
3332—17.....	Tinged blue	504—18	—	36	15	51
	»	505—18	—	19	6	25
	»	506—18	—	61	20	81
	»	507—18	—	17	3	20
3334—17.....	»	524—18	—	46	12	58
		Total	—	179	56	235
3332—17.....	White	501—18	—	—	15	15
	»	502—18	—	—	25	25
		Total	—	—	40	40

TABLE 16. F_2 of the cross violet $\times$ white n.r 6.

Colour of parent plant	Field-number	Blue	Violet	White	Total
Blue	3337—17	4	3	1	8
»	3338—17	23	13	7	43
	Total	27	16	8	51

TABLE 17. F_3 of the cross violet $\times$ white n:r 6.

Number of parent family	Colour of parent plant	Field-number	Blue	Violet	White	Total
3338-17.....	Blue	556-18	35	10	14	59
	»	558-18	33	6	7	46
	»	559-18	15	1	10	26
	»	561-18	44	23	19	86
	»	562-18	89	36	42	167
	Total		216	76	92	384
3338-17.....	Blue	557-18	56	28	—	84
	»	560-18	25	6	—	31
	»	565-18	25	6	—	31
	»	568-18	29	10	—	39
	Total		135	50	—	185
3338-17.....	Blue	563-18	48	—	16	64
	»	564-18	48	—	13	61
	»	566-18	54	—	10	64
	»	567-18	19	—	4	23
	Total		169	—	43	212
3338-17.....	Violet	572-18	—	74	—	74
	»	573-18	—	54	—	54
	»	574-18	—	98	—	98
	Total		—	226	—	226
3337-17.....	Violet	554-18	—	15	8	23
3338-17.....	»	570-18	—	53	16	69
	»	571-18	—	68	31	99
	»	575-18	—	81	23	104
	»	576-18	—	25	15	40
	Total		—	242	93	335
3338-17.....	White	577-18	—	—	36	36

TABLE 18. F_2 of the cross bluish red $\times$ white n:r 7.

Colour of parent plant	Field-number	Blue	Bluish red	White	Total
Blue	545-19	10	3	4	17
»	546-19	31	12	13	56
	Total	41	15	17	73

TABLE 19. F_3 of the cross bluish red $\times$ white n:r 7.

Number of parent family	Colour of parent plant	Field-number	Blue	Bluish red	White	Total
546-19	Blue »	338-20	42	—	—	42
		345-20	51	—	—	51
		Total	93	—	—	93
	Blue » » » »	342-20	22	7	8	37
		346-20	17	7	7	31
		348-20	18	2	7	27
		354-20	20	9	10	39
		355-20	23	5	10	38
		Total	100	30	42	172
	Blue »	337-20	17	9	—	26
		347-20	16	5	—	21
		Total	33	14	—	47
	Blue »	344-20	33	—	13	46
		352-20	14	—	11	25
		Total	47	—	24	71
	Bluish red » » »	358-20	—	21	—	21
		359-20	—	36	—	36
		360-20	—	25	—	25
		363-20	—	35	—	35
		Total	—	117	—	117
	Bluish red	361-20	—	33	7	40
	White. » » » » » »	228-20	—	—	7	7
		229-20	—	—	8	8
		231-20	—	—	21	21
		232-20	—	—	17	17
		233-20	—	—	14	14
		234-20	—	—	15	15
		236-20	—	—	12	12
		Total	—	—	94	94

TABLE 20. F_2 of the cross tinged blue $\times$ violet n:r 8.

Colour of parent plant	Field-number	Blue	Violet	Tinged blue	Total
Slightly diluted blue	618-18	14	11	14	39
	619-18	32	12	13	57
	620-18	31	24	18	73

Colour of parent plant	Field-number	Blue	Violet	Tinged blue	Total
Slightly diluted blue	621—18	16	7	12	35
	622—18	45	29	30	104
	623—18	75	31	38	144
	624—18	44	14	17	75
	625—18	53	19	26	98
	626—18	50	21	33	104
	627—18	26	16	10	52
	628—18	32	34	19	85
	629—18	52	23	25	100
Total		470	241	255	966

TABLE 21. F_3 of the cross tinged blue $\times$ violet n:r 8.

Number of parent family	Colour of parent plant	Field-number	Blue	Violet	Tinged blue	Total
619—18	Blue	329—19	23	8	7	38
	»	330—19	34	15	18	67
	»	331—19	9	5	11	25
620—18	»	336—19	15	11	9	35
	»	339—19	19	13	14	46
	»	346—19	43	12	15	70
	»	347—19	28	20	14	62
	»	348—19	25	12	16	53
	»	349—19	38	18	8	64
	»	350—19	39	12	20	71
621—18	»	351—19	14	5	11	30
	»	355—19	23	12	17	52
	»	356—19	25	6	11	42
	»	357—19	14	6	8	28
	»	359—19	21	12	11	44
	»	360—19	28	17	17	62
	»	361—19	34	25	9	68
622—18	»	381—19	15	3	7	25
	»	384—19	22	14	9	45
	»	387—19	21	4	12	37
	»	389—19	25	13	9	47
	»	390—19	22	8	11	41
	»	391—19	18	7	9	34
	»	392—19	19	9	12	40
623—18	»	405—19	17	9	10	36
	»	409—19	7	3	10	20
	»	410—19	15	9	11	35
	»	411—19	15	11	12	38

Number of parent family	Colour of parent plant	Field-number	Blue	Violet	Tinged blue	Total
623—18	Blue	413—19	15	11	3	29
	»	414—19	25	3	2	30
	»	416—19	11	4	5	20
	»	417—19	16	13	7	36
	»	418—19	7	6	7	20
	»	419—19	14	12	10	36
	»	420—19	17	9	12	38
	»	434—19	8	9	8	25
	»	436—19	12	7	9	28
	»	437—19	22	12	10	44
624—18	»	438—19	19	7	10	36
	»	439—19	21	12	9	42
	»	440—19	17	10	9	36
	»	441—19	16	13	10	39
	»	442—19	20	8	12	40
	»	443—19	28	13	11	52
	»	444—19	7	7	12	26
	»	445—19	20	12	15	47
	»	447—19	11	3	7	21
	»	465—19	16	10	11	37
625—18	»	466—19	14	8	5	27
	»	467—19	16	5	6	27
	»	468—19	16	6	12	34
	»	469—19	9	8	15	32
626—18	»	483—19	30	18	8	56
628—18	»	490—19	15	9	2	26
	»	491—19	12	6	5	23
	»	492—19	20	11	3	34
629—18	»	498—19	16	9	12	37
	»	500—19	10	6	7	23
	»	501—19	29	8	10	47
	»	503—19	23	12	13	48
Total			1,160	586	605	2,351
620—18	Violet	341—19	—	63	—	63
621—18	»	374—19	—	63	—	63
	»	376—19	—	39	—	39
622—18	»	380—19	—	30	—	30
	»	394—19	—	37	—	37
	»	395—19	—	56	—	56
623—18	»	421—19	—	33	—	33
	»	422—19	—	38	—	38
	»	423—19	—	37	—	37
	»	424—19	—	49	—	49
	»	425—19	—	45	—	45

Number of parent family	Colour of parent plant	Field-number	Blue	Violet	Tinged blue	Total
623-18	Violet	426-19	—	33	—	33
	»	448-19	—	44	—	44
624-18	»	449-19	—	58	—	58
	»	450-19	—	38	—	38
	»	452-19	—	50	—	50
625-18	»	472-19	—	20	—	20
626-18	»	484-19	—	38	—	38
628-18	»	493-19	—	24	—	24
	»	495-19	—	43	—	43
629-18	»	506-19	—	22	—	22
	»	507-19	—	56	—	56
		Total	—	916	—	916
618-18	Tinged blue	327-19	—	—	20	20
620-18	»	344-19	—	—	34	34
	»	345-19	—	—	33	33
	»	362-19	—	—	62	62
	»	369-19	—	—	55	55
621-18	»	370-19	—	—	73	73
	»	371-19	—	—	34	34
	»	372-19	—	—	34	34
	»	373-19	—	—	47	47
	»	396-19	—	—	42	42
	»	397-19	—	—	36	36
	»	399-19	—	—	24	24
622-18	»	400-19	—	—	30	30
	»	401-19	—	—	42	42
	»	402-19	—	—	37	37
	»	403-19	—	—	25	25
	»	404-19	—	—	42	42
	»	428-19	—	—	24	24
	»	429-19	—	—	29	29
623-18	»	430-19	—	—	40	40
	»	431-19	—	—	38	38
	»	432-19	—	—	27	27
	»	433-19	—	—	35	35
	»	455-19	—	—	40	40
	»	456-19	—	—	48	48
	»	457-19	—	—	22	22
624-18	»	458-19	—	—	49	49
	»	459-19	—	—	36	36
	»	460-19	—	—	44	44
	»	461-19	—	—	34	34
	»	462-19	—	—	39	39
	»	463-19	—	—	35	35

Number of parent family	Colour of parent plant	Field-number	Blue	Violet	Tinged blue	Total
624—18	Tinged blue	464—19	—	—	30	30
625—18	»	473—19	—	—	39	39
	»	474—19	—	—	54	54
	»	475—19	—	—	66	66
	»	476—19	—	—	26	26
	»	477—19	—	—	31	31
626—18	»	479—19	—	—	20	20
	»	486—19	—	—	33	33
628—18	»	496—19	—	—	55	55
	»	497—19	—	—	26	26
629—18	»	509—19	—	—	30	30
	»	512—19	—	—	42	42
Total			—	—	1,662	1,662

TABLE 22. F_2 of the cross bluish red $\times$ violet n:r 9.

Colour of parent plant	Field-number	Blue	Violet	Bluish red	Pure red	Total
Blue	597—18	55	24	18	5	102
»	598—18	38	14	16	—	68
»	599—18	27	12	9	—	48
»	600—18	14	5	3	—	22
»	601—18	18	6	9	—	33
»	602—18	27	14	12	1	54
»	603—18	62	27	27	—	116
»	604—18	15	9	6	1	31
»	605—18	64	43	27	2	136
»	606—18	77	30	35	4	146
»	607—18	30	20	20	1	71
»	608—18	67	45	23	—	135
»	609—18	62	20	21	1	104
		556	269	226	15	1,066

TABLE 23. F_3 of the cross bluish red $\times$ violet n:r 9.

Number of parent family	Colour of parent plant	Field-number	Blue	Violet	Bluish red	Pure red	Total
598—18 ...	Blue	20—19	35	5	2	3	45
603—18 ...	»	73—19	37	4	5	13	59
605—18 ... {	»	92—19	49	4	8	9	70
	»	96—19	39	8	10	14	71

Number of parent family	Colour of parent plant	Field- number	Blue	Violet	Bluish red	Pure red	Total
605-18 ...	Blue	112-19	49	5	2	10	66
607-18 ...	»	189-19	32	2	3	13	50
		Total	241	28	30	62	361
598-18 ...	Blue	16-19	26	17	19	—	62
	»	17-19	39	18	10	—	67
	»	18-19	32	18	12	1	63
	»	19-19	19	12	10	—	41
601-18 ...	»	24-19	24	5	12	—	41
	»	41-19	38	15	30	—	83
	»	44-19	21	2	5	—	28
	»	45-19	15	11	10	—	36
602-18 ...	»	46-19	26	15	8	2	51
	»	47-19	28	19	16	—	63
	»	55-19	25	7	9	—	41
	»	65-19	28	18	20	—	66
603-18 ...	»	66-19	19	6	10	—	35
	»	68-19	15	5	6	—	26
	»	74-19	30	14	11	1	56
	»	84-19	29	17	13	2	61
604-18 ...	»	90-19	32	8	18	—	58
	»	93-19	27	17	16	—	60
	»	99-19	28	10	17	1	56
	»	100-19	11	8	5	—	24
605-18 ...	»	104-19	45	10	12	—	67
	»	105-19	30	18	17	—	65
	»	107-19	30	17	17	1	65
	»	108-19	22	14	13	—	49
606-18 ...	»	113-19	15	8	15	—	38
	»	115-19	13	4	8	1	26
	»	154-19	25	13	5	—	43
	»	156-19	7	8	10	—	25
607-18 ...	»	164-19	14	7	3	—	24
	»	165-19	27	15	14	1	57
	»	166-19	41	9	18	—	68
	»	168-19	38	12	14	1	65
608-18 ...	»	169-19	35	15	18	3	71
	»	190-19	32	15	6	—	53
	»	201-19	45	17	12	1	75
	»	203-19	35	15	12	1	63
609-18 ...	»	204-19	28	17	15	1	61
	»	205-19	29	15	18	1	63
	»	207-19	30	14	10	—	54
	»	212-19	7	4	9	—	20
609-18 ...	»	237-19	31	15	13	2	61

Number of parent family	Colour of parent plant	Field- number	Blue	Violet	Bluish red	Pure red	Total
609—18 ... {	Blue	239—19	28	25	14	—	67
	»	241—19	23	11	12	—	46
		Total	1,142	540	542	20	2,244
598—18 ... {	Blue	23—19	34	10	—	—	44
	»	25—19	73	27	—	—	100
603—18 ... {	»	64—19	52	13	—	—	65
	»	71—19	19	6	—	—	25
605—18 ... {	»	95—19	39	6	—	—	45
	»	97—19	46	5	—	—	51
	»	110—19	35	15	—	—	50
	»	119—19	38	9	—	—	47
	»	158—19	18	6	—	—	24
606—18 ... {	»	161—19	24	3	—	—	27
	»	162—19	36	17	—	—	53
	»	167—19	55	15	—	—	70
	»	172—19	20	6	—	—	26
	»	193—19	17	4	—	—	21
607—18 ... {	»	194—19	33	15	—	—	48
608—18 ... {	»	202—19	18	13	—	—	31
609—18 ... {	»	238—19	33	15	—	—	48
	»	242—19	29	4	—	—	33
		Total	619	189	—	—	808
599—18 ... {	Blue	33—19	39	—	13	—	52
	»	34—19	28	—	9	—	37
600—18 ... {	»	36—19	66	—	36	—	102
601—18 ... {	»	43—19	23	—	11	—	34
603—18 ... {	»	67—19	28	—	11	—	39
	»	72—19	37	—	16	—	53
	»	91—19	27	—	8	—	35
605—18 ... {	»	98—19	42	—	8	—	50
	»	101—19	48	—	6	—	54
	»	102—19	46	—	14	—	60
	»	103—19	25	—	11	—	36
	»	106—19	36	—	11	—	47
606—18 ... {	»	109—19	39	—	16	—	55
	»	118—19	36	—	9	—	45
	»	157—19	30	—	16	—	46
	»	159—19	54	—	17	—	71
	»	160—19	30	—	12	—	42
608—18 ... {	»	206—19	28	—	15	—	43
609—18 ... {	»	240—19	33	—	10	—	43
		Total	695	—	249	—	944

Number of parent family	Colour of parent plant	Field- number	Blue	Violet	Bluish red	Pure red	Total
602—18 ...	Violet	57—19	--	59	—	—	59
	"	58—19	.	20	—	—	20
	"	59—19	.	43	—	—	43
	"	60—19	—	63	—	—	63
603—18 ...	"	75—19	—	72	—	—	72
604—18 ...	"	86—19	—	58	—	—	58
	"	121—19	—	79	—	—	79
	"	122—19	—	54	—	—	54
	"	132—19	—	60	—	—	60
605—18 ...	"	133—19	—	22	—	—	22
	"	136—19	—	63	—	—	63
	"	137—19	—	67	—	—	67
	"	138—19	—	54	—	—	54
606—18 ...	"	140—19	—	68	—	—	68
	"	141—19	—	45	—	—	45
	"	176—19	—	23	—	—	23
	"	177—19	—	37	—	—	37
607—18 ...	"	196—19	—	42	—	—	42
	"	214—19	—	51	—	—	51
	"	216—19	—	68	—	—	68
	"	218—19	—	47	—	—	47
608—18 ...	"	219—19	—	22	—	—	22
	"	221—19	—	81	—	—	81
	"	222—19	—	54	—	—	54
	"	223—19	—	77	—	—	77
609—18 ...	"	227—19	—	44	—	—	44
	"	244—19	—	66	—	—	66
	"	245—19	—	52	—	—	52
Total			—	1,491	—	—	1,491
600—18 ...	Violet	39—19	—	56	—	18	74
601—18 ...	"	48—19	—	24	—	11	35
	"	49—19	—	39	—	10	49
	"	120—19	—	52	—	21	73
	"	123—19	—	53	—	21	74
605—18 ...	"	128—19	—	40	—	12	52
	"	129—19	—	47	—	19	66
	"	131—19	—	24	—	4	28
	"	134—19	—	31	—	16	47
606—18 ...	"	135—19	—	37	—	14	51
	"	139—19	—	18	—	6	24
	"	174—19	—	26	—	8	34
	"	195—19	—	30	—	10	40
608—18 ...	"	213—19	—	45	—	10	55

Number of parent family	Colour of parent plant	Field- number	Blue	Tinged blue	Bluish red	Tinged red	Total
608—18 ...	Violet	217—19	—	45	—	14	59
»		220—19	—	53	—	10	63
609—18 ...		224—19	—	43	—	12	55
»		247—19	—	16	—	4	20
		Total	—	679	—	220	899
598—18 ...	Bluish red	29—19	—	—	60	—	60
»		32—19	—	—	69	—	69
601—18 ...	»	51—19	—	—	76	—	76
»	»	61—19	—	—	34	—	34
602—18 ...	»	62—19	—	—	39	—	39
»	»	63—19	—	—	31	—	31
»	»	78—19	—	—	63	—	63
603—18 ...	»	79—19	—	—	94	—	94
»	»	82—19	—	—	73	—	73
»	»	83—19	—	—	47	—	47
604—18 ...	»	88—19	—	—	23	—	23
»	»	142—19	—	—	71	—	71
»	»	144—19	—	—	55	—	55
»	»	146—19	—	—	39	—	39
605—18 ...	»	148—19	—	—	28	—	28
»	»	149—19	—	—	33	—	33
»	»	150—19	—	—	54	—	54
»	»	151—19	—	—	54	—	54
»	»	152—19	—	—	63	—	63
»	»	178—19	—	—	37	—	37
»	»	179—19	—	—	53	—	53
606—18 ...	»	181—19	—	—	69	—	69
»	»	186—19	—	—	51	—	51
»	»	187—19	—	—	65	—	65
607—18 ...	»	197—19	—	—	29	—	29
»	»	228—19	—	—	49	—	49
»	»	231—19	—	—	60	—	60
608—18 ...	»	234—19	—	—	66	—	66
»	»	236—19	—	—	57	—	57
»	»	250—19	—	—	58	—	58
609—18 ...	»	251—19	—	—	68	—	68
»	»	252—19	—	—	61	—	61
		Total	—	—	1,729	—	1,729
598—18 ...	Bluish red	28—19	—	—	58	14	72
599—18 ...		35—19	—	—	35	12	47
»		80—19	—	—	15	7	22
603—18 ...		81—19	—	—	34	6	40
605—18 ...	»	143—19	—	—	40	14	54

Number of parent family	Colour of parent plant	Field-number	Blue	Violet	Bluish red	Pure red	Total
605-18 ...	Bluish red	145-19	—	—	49	20	69
	»	147-19	—	—	30	10	40
606-18 ...	»	180-19	—	—	55	11	66
	»	182-19	—	—	29	13	42
607-18 ...	»	200-19	—	—	17	6	23
608-18 ...	»	229-19	—	—	55	10	65
	»	230-19	—	—	47	17	64
609-18 ...	»	249-19	—	—	43	17	60
		Total	—	—	507	157	664
605-18 ...	Pure red	153-19	—	—	—	10	10
606-18 ...	»	188-19	—	—	—	63	63
		Total	—	—	—	73	73

TABLE 24. F_4 of the cross bluish red $\times$ violet n:r 9.

Number of parent family	Colour of parent plant	Field-number	Blue	Violet	Bluish red	Pure red	Total
92-19	Blue	299-20	22	1	4	2	29
	»	301-20	26	8	4	2	40
	»	302-20	22	3	4	6	35
	»	312-20	23	2	2	3	30
	»	315-20	27	2	4	4	37
	»	319-20	25	1	4	5	35
	»	321-20	18	5	5	1	29
	»	322-20	23	5	4	4	36
	»	326-20	18	1	1	6	26
	Total		204	28	32	33	297
92-19	Blue	324-20	9	8	3	—	20
	Blue	303-20	22	—	—	—	22
	»	309-20	32	—	—	—	32
	»	310-20	23	—	—	—	23
	»	314-20	24	—	—	—	24
	»	316-20	46	—	—	—	46
	»	317-20	50	—	—	—	50
	Total		197	—	—	—	197
	Blue	292-20	29	5	—	—	34
	»	300-20	21	5	—	—	26
	»	313-20	30	9	—	—	39
	»	318-20	19	2	—	—	21
		Total	99	21	—	—	120

Number of parent plant	Colour of parent plant	Field-number	Blue	Violet	Bluish red	Pure red	Total
92-19	Blue »	311-20	19	—	9	—	28
		325-20	20	—	6	—	26
		Total	39	—	15	—	54
	Violet	327-20	—	24	—	—	24
	Violet	328-20	—	20	—	6	26
	Bluish red » » »	330-20	—	—	31	—	31
		331-20	—	—	21	—	21
		332-20	—	—	60	—	60
		335-20	—	—	47	—	47
		Total	—	—	159	—	159
	Bluish red	334-20	—	—	18	5	24

TABLE 25. F_2 of the cross bluish red $\times$ tinged blue n:r 10.

Colour of parent plant	Field-number	Blue	Tinged blue	Bluish red	Tinged red	Total
Slightly diluted blue	614-18	8	11	8	2	29
	615-18	22	9	12	2	45
	616-18	47	12	21	2	82
	Total	77	32	41	6	156

TABLE 26. F_3 of the cross bluish red $\times$ tinged blue n:r 10.

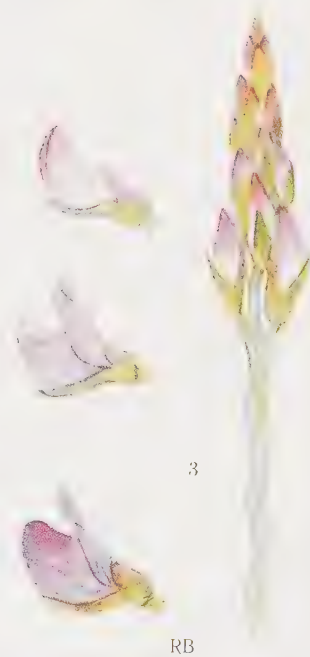
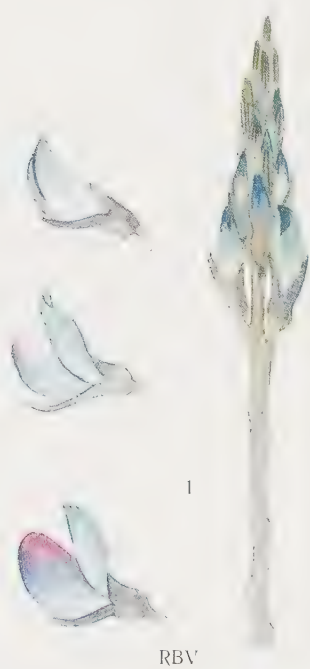
Number of parent family	Colour of parent plant	Field- number	Blue	Tinged blue	Bluish red	Tinged red	Total
616—18 ... {	Blue »	303—19	26	—	—	—	26
		305—19	38	—	—	—	38
		Total	64	—	—	—	64
615—18 ... {	Blue » » » »	286—19	18	6	10	—	34
		287—19	28	2	6	2	38
		298—19	38	15	9	—	62
		299—19	16	6	4	1	27
		301—19	29	18	13	—	60
616—18 ... {	Sl. dil. blue » » » » »	306—19	28	14	15	—	57
		307—19	35	23	17	3	78
		309—19	21	8	9	—	38
		312—19	21	6	7	—	34
		313—19	9	8	4	1	22
		315—19	34	15	8	—	57
		Total	277	121	102	7	507

Number of parent family	Colour of parent plant	Field- number	Blue	Tinged blue	Bluish red	Tinged red	Total
616—18 ...	Sl. dil. blue	311—19	66	19	—	—	85
614—18 ...	Blue	279—19	22	—	9	—	31
615—18 ...	»	288—19	20	—	5	—	25
616—18 ...	»	302—19	19	—	5	—	24
		304—19	17	—	6	—	23
		Total	78	—	25	—	103
615—18 ...	Tinged blue	291—19	—	22	—	—	22
	»	293—19	—	43	—	—	43
		Total	—	65	—	—	65
616—18 ...	Tinged blue	316—19	—	48	—	21	69
	»	317—19	—	60	—	24	84
		Total	—	108	—	45	153
616—18 ...	Bluish red	318—19	—	—	33	—	33
	»	322—19	—	—	51	—	51
		Total	—	—	84	—	84
614—18 ...	Bluish red	283—19	—	—	14	6	20
615—18 ...	Tinged red	297—19	—	—	—	3	3

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CARL HALLQVIST: LUPINUS.



1. Blue. 2. Violet. 3. Bluishred. 4. Pure red.



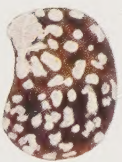
5

RBVf



6

RBf



7



8



9



10



11



G. Hansen pinx.

5. Tinged blue. 6. Tinged red. 7. Earth-brown, non-marbled. 8. Earth-brown, marbled.
9. Rust-brown, non-marbled. 10. Rust-brown, marbled. 11. White.

